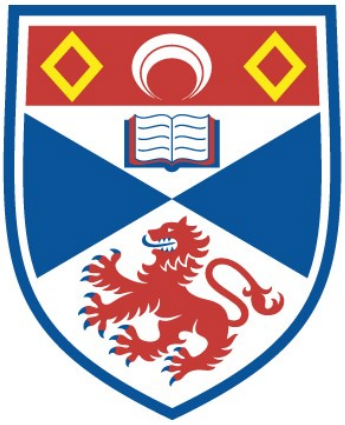
A STUDY OF PUCCINIA POLYSORA UNDERWOOD IN WEST AFRICA

Robert Hendry Cammack

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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A STUDY OF *PUCCINIA POLYSORA* UNDERWOOD IN WEST AFRICA.

by

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A Thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

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Career

I first matriculated in the University of St. Andrews in October 1948 and graduated B.Sc. with Second Class Honours in Botany in June 1952.

In December 1952 I was appointed Temporary Scientific Officer, Mycologist (on Probation), Colonial Research Service, and was posted to the West African Maize Research Unit at Ibadan, Nigeria.

In December 1954 I was offered a permanent appointment in the Colonial Research Service as Scientific Officer (Mycologist).

In October 1954 I was admitted as a research student in the Faculty of Science, in the Department of Botany of St. Salvator's College, University of St. Andrews.

Declaration

I hereby declare that the following Thesis is based on the record of work done by me, that the Thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The research was carried out at the West African Maize Research Unit, Federal Department of Agricultural Research, Moor Plantation, Ibadan, Nigeria and in the Department of Botany at St. Salvator's College of the University of St. Andrews under the direction of Dr. J.A. Macdonald.

Certificate

I certify that Robert H. Cammack has spent nine terms of research under my direction and that he has fulfilled the conditions of Ordinances 16 and 61 (St. Andrews) and that he is qualified to submit the following Thesis for the Degree of Doctor of Philosophy.

Acknowledgements

I wish to record my indebtedness to the following:-

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A STUDY OF *PUCCINIA POLYSORA* UNDERW. IN WEST AFRICA.

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SECTION 1.

Summary

1. The incidence and extent of fungal diseases on *Zea mays* L. in West Africa are briefly described.

2. The terms of reference of the West African Maize Research Unit are given in relation to research on *Puccinia polysora*, a leaf rust of *Zea mays*.

3. In West Africa *P. polysora* is responsible for a major economic loss conservatively estimated as £5 - £7.5 million sterling per annum.

4. The various possible methods of introduction of *P. polysora* into West Africa are discussed and it is suggested that the fungus was introduced as viable uredospores on unhusked ears of maize flown into West Africa from the Americas.

1. INTRODUCTION.

The estimated production of maize, Zea mays L., in the British West African Territories according to the World Census of Agriculture (1950) is given in Table 1. Further details are given by the Nigerian Survey Department (1950) and by Prest and Stewart (1953), all these estimates being obtained from the same sample survey. It is admitted by Prest and Stewart (loc.cit.) that maize yields are "Category 'C'" and as such are subject to a standard error of 25%. From the estimated figures in Table 1 it is evident that any reduction in the production of maize due to diseases and pests may involve the four territories in a financial loss of several million pounds sterling per annum. In Table 2 the first three organisms named are the main identifiable causes of loss in yield. To these must be added a further loss due to lesser pathogens and pests. Table 2 gives an analysis of loss in yield in the different vegetation zones (Keay, 1953) of the British West African Territories together with an aggregated average zonal loss for all pests and diseases. The information used in these estimations is based on a variety of sources. Bowden (1956, unpub.) has contributed to the study of stem borers, Busseola and Sesamia spp., and lossess caused by them in Ghana and Nigeria. For rust, Puccinia polysora, reference has been made to the work of Blane (1952) in Ghana, and that of the writer in Nigeria.

The relationship between rust intensity and yield is reported in the Annual Report of the West African Maize Research Unit, 1953. Further studies have been made in East Africa by Ellis (1954) and Guinard and Bates (personal communications). Provisional estimates of the losses due to Helminthosporium turcicum Pass., leaf blight of maize, have been obtained from experiments in chemical control conducted by the writer. The main evidence, however, is available from other countries since extensive data is not yet available from West Africa on the inter- and intrazonal variation in the intensity of these diseases. Referring to the aggregate values given at the bottom of Table 2 it is estimated that the average annual loss in yield in the field due to pests and diseases is approximately 30%. Relating this figure to the calculated value of maize production in Table 1, and based on the world market price of £27 per ton, this represents an annual loss of some £7,500,000.

Table 1./

Table 1.

The annual cash value of maize, Zea mays L., in the British West African Territories. Estimates based on the world market price of £27 per ton dry grain and subject to an error of estimation of ± 25%.

Territory	Production per annum in tons.	Percentage total grain produced.	Approximate Value 1955 (Standard Error ± 25%)
Nigeria	743,000	19.3	£20,061,000
Ghana	163,000	45.2	£ 4,401,000
Sierra Leone	8,000	2.6	£ 216,000
Gambia	1,500	3.3	£ 40,500
TOTAL	915,500		£24,718,000

Table 2.

Estimated losses in maize yields in the various vegetation zones of the British West African territories due to pests and diseases.

Organism	Northern Guinea Savannah	Southern Guinea Savannah	Transitional Rain Forest.	Rain Forest
Stem Borers Busseola, Sesamia etc.	15%	20%	10-20%	10-20%
	(Exceptional 90% losses have been recorded where maize development has coincided with peak emergence of moths)			
Puccinia polysora	5%	5-20%	20-40%	30-40%
	(Complete crop failures have been reported)			
Leaf Blights Helminthosporium turcicum etc.	5%	10-20%	10-20%	15-20%
Other Pests and Diseases	30%	20%	20-40%	20-40%
	(Ear worms, ear rots, weevils, etc.)			

It is significant that the maize growing areas of West Africa, in the rain forest and southern Guinea savannah zones (Keay, 1953), coincide with the areas of high population and relatively high standard of living. It is likely that the production of maize will increase, especially as the yield per annum per acre of grain, subject to controlled breeding and agronomic methods, could be higher for maize than any other local cereal. This loss is, therefore, likely to increase rather than diminish unless disease control methods are employed and resistant varieties introduced.

Fungal diseases of maize in West Africa are numerous and widely distributed. Lists of fungi have been prepared by Deighton (1936) in Sierra Leone; Saccas (1952) in French Equatorial Africa; Bunting (1927) and Hughes (1952, 1953) in Ghana, and a preliminary list by West (1938) in Nigeria.

During 1953-55 a maize disease survey was carried out by the writer in Nigeria. The system of rust assay plots described in Section 5.2 was used to assess the occurrence and distribution of maize diseases and with the assistance of members of staff of the respective agricultural stations a collection of specimens of infected maize was made from the assay plots distributed throughout the maize growing areas of Nigeria. A list of fungi is given in Appendix 1. Preliminary culturing of the isolates was carried out by the writer at Ibadan and identifications confirmed by the Commonwealth Mycological Institute. Type specimens of most

of the collection were retained by the Institute.

The disease survey demonstrated the large number of maize pathogens present in Nigeria and subsequent collections showed most of the pathogens from the Nigerian collection to be present in other parts of the West African coast. The incidence of these diseases was highest in the coastal rain forest areas and generally less severe in the northern areas. The West African coastal areas are subject to high rainfall, 80 - 150", high temperatures around 80°F with a small diurnal range and consistantly high humidity with extended dew periods. These conditions encourage the development and spread of many of the major maize diseases. In the interior the rainfall is markedly less, 50 - 25", and temperatures much higher than on the coast, with a greater diurnal range. Humidity is low, with short dew periods. These factors reduce the incidence of disease and will be more fully discussed in Section 5.

Methods of husbandry adopted by the local farmer in West Africa are primitive. A system of 'growth to exhaustion' is practised in which an area of forest is cleared and often planted with successive crops of maize. Debris is seldom cleared before the second planting and rotting stalks and leaves serve as sources of inoculum allowing subsequent infection of the new crop. As cropping proceeds no fertilisers are applied and poor, weakened growth increases the proneness of the maize crop to disease.

In 1949, a leaf rust of maize, Puccinia polysora Underw. was reported as a new rust of maize in West Africa and in the space of a few years had spread over the entire tropical belt of Africa, from Sierra Leone in the west to the Indian Ocean. The severity of the new disease was such that the Colonial Office, with the co-operation of the four British West African Governments formed the West African Maize Rust Research Unit in 1951, a team consisting of a geneticist-plant breeder and a mycologist to assess the incidence and, if possible, combat the disease by introducing and breeding resistant varieties of Zea mays. The breeding section of the Unit is concerned with the selection of local and introduced maize varieties for resistance to Puccinia polysora and the improvement of yielding ability under prevailing local conditions in West Africa. The Plant Pathology section is responsible for the study of the behaviour of the fungus and the development of field and greenhouse techniques to support the breeding programme.

The following sections describe and discuss the work undertaken by the writer on aspects of the biology and epidemiology of the rust in West Africa, and on field and greenhouse studies in relation to the work of the Unit in securing resistance to the disease.

2. THE ECONOMIC EFFECT OF THE DISEASE IN WEST AFRICA.

In West Africa there was a 'rather severe' outbreak of rust in the Bo district of Sierra Leone (see map) in 1949, the first attributable to Puccinia polysora in Africa (Deighton, 1949). It was widespread in Sierra Leone in 1950 and 1951 but, since most of the maize is harvested green in that territory, losses were light.

Meiffren (1950) states that in 1950 the main maize growing areas of Pobe and Allada in Dahomey were severely attacked early in the season. Later that year the regions of Saketé, Adjohom, the outskirts of Porto Novo and Athieme showed fields of maize a bright red colour due to the intensity of rust. The disease was at its peak from May until September and caused serious local food shortages. The market price of maize rose from fr 40 in May to fr 200 in September as opposed to an average increase in cost of 10% over the equivalent period in previous years prior to the outbreak of the epidemic. Severe losses were also experienced in the Ivory Coast where, in some instances, total loss of crops occurred. The rust first appeared in Ghana in May 1950 in the Colony and Ashanti regions of the south, and in Trans-Volta in June. Rust attack on the early sown crops became serious only after the ears had set, with the result that losses were small. Late planted maize (i.e. April) was attacked at the flowering stage and losses were estimated at 50%. Rust was also

reported in the Northern territories of Ghana but in no case was the attack so severe as in the Southern coastal areas. The second season crop, usually planted in early September and harvested in late November, was less affected in all areas than the first season crop. The following year, 1951, the rust appeared in the coastal regions of Ghana in April but was not observed inland until May. Farmers in Togoland and Trans-Volta replaced maize with cow peas and ground nuts, fearing a repetition of the 1950 losses. In the first season planting, 1951, early sown crops (February-March) showed a small reduction in yield owing to the onset of rust after the ears had set but in later plantings (April-May) the average loss throughout the maize areas was estimated at 40%. In the coastal savannah areas of the Accra Plain and the Volta, yields were reported as 'very poor' and the grain small and shrunken. Yields in the coastal rain forest regions were estimated as being reduced by 60%. In the drier savannah areas in the northern region of the territory rust attack was reported to be light and losses small. The second season crop, 1951, was attacked generally with average losses of 30% but, as in 1950, losses were less than in the first season plantings. In Nigeria, records of losses in yields are few or completely lacking during the early years of the epiphytotic. It appears that, although losses during 1950 and 1951 were considerable, they were less than those reported

in Ghana in these years. Casual reports stated that damage ranged from estimated losses of 70% in the south-west corner of the Colony to insignificant losses in other regions. In 1952 the entire southern region of Nigeria was attacked early in the first growing season and by the flowering stage maize plots were a conspicuous red colour, losses being estimated at 60-70%. As in Ghana the drier areas in the central belt of the colony were less severely affected by the disease.

Estimates of the losses in yield in the early stage of the epidemic were based on preliminary trials at Government Agricultural Stations in Nigeria and Ghana, using copper sprays and sulphur dust. These are fully described in memoranda by the Department of Agriculture, Nigeria (1952), Department of Agriculture, Ghana (1952) and Blane (1953). These trials were conducted under husbandry methods not normally available to the farmer and, as a result, possibly underestimate the actual losses. The following Table gives the average percentage losses, based on the results of the fungicide trials, in the different territories. No trials were made in Sierra Leone and no figures are available.

Table 3.

The estimated average percentage losses in yield of maize in dry grain weight in Nigeria and Ghana.

	1950	1951	1952	1953	1954	1955	1956
Nigeria	?	60%	60%	?	40%	?	38%
Ghana	50%	60%	50%	?	?	?	?

When the percentage losses are related to the estimated production of grain (World Census of Agriculture, 1950), and valued at the world market price of £27 per ton dry grain, they represent an annual loss of some £10.25 - £14.5 million. Stanton (1957) points out, however, that since maize is one of a number of alternative foodstuffs in the territories mentioned, a more realistic figure, in terms of the internal economy of the country, would be that of the cost of production 'ex-farms'. The figure would then be reduced to about £15 per ton dry grain.

On the basis of these local values it is estimated that the rust causes a total annual loss to the colonies of Sierra Leone and Nigeria and the State of Ghana of approximately £5,000,000 - £7,500,000.

A CONSIDERATION OF THE METHOD OF INTRODUCTION OF PUCCINIA POLYSORA INTO THE AFRICAN CONTINENT.

Whenever a new apiphytotic breaks out, questions arise as to whether or not it is in fact caused by a new pathogen or by one which has been present previously and overlooked, and secondly, if it is a disease new to the affected area how was it introduced?

Several workers have suggested that P. polysora was not introduced into West Africa in 1949, but was, in fact, over-

looked and at that time suddenly assumed a more virulent form. P. polysora bears strong superficial resemblance to a second rust of Zea mays, Puccinia sorghi (Cammack, 1955) and prior to 1949 this latter rust had been reported in twelve territories in the African Continent. After Cummins (1941) had identified P. polysora on Zea mays in America all collections of maize rusts at the Commonwealth Mycological Institute were re-examined. These included all available specimens from Africa and in 1944 it was established that only P. sorghi was present in the African Continent. On the severe outbreak of rust in Sierra Leone in 1949 specimens collected in the colony were identified as P. polysora (Deighton, 1949). Specimens collected the same year in Ghana were, without exception, P. sorghi, whereas P. polysora did not appear in that colony until the following year, 1950. All available evidence based on herbarium collections and field observations points to the introduction of the rust in or about 1949, and none supports the theory that the fungus was present previously as a weak parasite of maize. Some possible methods of entry of the rust into the African Continent are discussed.

Introduction by Inoculum Carried in Wind Current.

Wind is the principal means of dissemination of the rusts both locally and over great distances. Schmidt (1925) estimated the absolute distance a uredospore could be carried in air currents to be 1,100 kilometres. This calculation was based on physical reasoning and had no supporting experimental

work. A better theoretical approach with a considerable amount of experimental evidence is that of Sutton (1932) and this is fully discussed by Gregory (1945). Stepanov (1935) calculated the absolute limit of dissemination of uredospores of Puccinia triticina as 1282 kilometres. Naumov (1939) calculated the theoretical limit of P. triticina to be 1200-12,000 kilometres. Moulton (1942) has illustrated the large overland distances travelled by rust spores in his study of black stem rust of wheat, which is carried northwards each year from Texas to Canada.

On release from the source, uredospores may be raised high in the atmosphere by thermal convection and frictional turbulence. Stackman et.al. (1923) trapped wheat leaf rust uredospores at a height of 16,000 - 16,500 ft. Height attained is of importance in the distance travelled from the source by uredospores in wind currents. Ukkelberg (1933) has shown theoretically that a wheat leaf rust uredospore at an elevation of 5,000 ft. in a 30 m.p.h. wind could travel a horizontal distance of 1,000 miles.

Little is known of the dispersal of fungus spores over large areas of water. Bisby (1935) and McCubbin (1944) have found spores present in small quantities over mid-Atlantic. Pady and Kapika (1955) exposed nutrient agar plates and silicone-coated slides at heights of 8,000 and 9,000 ft. respectively on two trans-Atlantic flights and found uredo-

spores to be comparatively rare, occurring as single uredospores on four slides exposed over eastern Canada and one on a slide exposed in a tropical air mass over Iceland.

It is known that prior to 1949 Puccinia polysora was confined to Central America and the West Indies. On the appearance of the rust in West Africa that year the source of infection was 3,500 miles distant and separated by the Atlantic Ocean.

Introduction by Seed Borne Inoculum.

The possibility of seed-borne infection, with special reference to wheat rust, received much attention from early workers and their theories have been reviewed by Levine (1919). Chester (1946) states that in severely rusted wheat crops the grain has been observed to be orange-tinted by the heavy covering of uredospores. Naumov (1939) maintains that it is unlikely that uredospores carried on grains would be capable of initiating infection of the young seedling, since the exposed tissues (coleoptiles, roots etc.) are not susceptible and by the time parts of the seedling prone to infection became available the spores have lost their viability or have germinated in the soil. Weber (1922) germinated maize and allowed the seedlings to grow until the radicle and coleoptile were approximately half an inch long and then immersed them in an aqueous suspension of uredospores of Puccinia sorghi for a period of five minutes. The seedlings were then planted two inches deep and four weeks later mature uredosori were

found 1.5 inches below the surface of the soil. The mechanical effect of rain splash could cause these sori to become exposed and afford a source of inoculum. There have been no reports of possible seed-borne transmission of P. polysora but Rhind (1952) has suggested the possibility of uredospores becoming lodged in the rudimentary glumes.

Several experiments were designed to investigate the possibility of seed-borne infection. These were carried out in a greenhouse at Ibadan using the rust susceptible variety of maize, Lagos White. Since all experiments gave negative results, only a brief description of the methods is given below.

I. The immersion of maize grains in a uredospore suspension.

a) Maize grains were washed in running water for 15 minutes and then immersed in a thick suspension of uredospores in distilled water for periods of 5, 15, and 60 minutes, 4 and 12 hours respectively. The batches of grain were removed from the suspension with forceps and planted one inch deep in sterilized sand culture.

b) Previously washed grains were soaked in sterile water for 24 hours and then immersed in a uredospore suspension for the same range of times as in I a), prior to being planted in sand culture.

II. The inoculation of the surface of maize grains.

a) Dry grains were rapidly washed with 70% alcohol, rinsed with sterile water and allowed to dry thoroughly before being heavily dusted with uredospores. Half the grains

were planted one inch deep and the rest two inches deep in sand culture with forceps.

b) Grains were washed in running water for fifteen minutes, rinsed in sterile water and then dipped in warm 1% agar. The surface of the agar was dusted with uredospores and the grains were then planted as in II a).

III. A repetition of the experiment by Weber (1922).

a) Maize grains were germinated until the coleoptiles were half an inch long. The seedlings were then immersed in a uredospore suspension for periods of 5, 15, and 60 minutes and planted 2 inches deep in sand culture.

b) Washed grains were soaked in sterile water until the coleoptiles began to grow. The seedlings were then dipped in warm 1% agar, dusted with uredospores and planted as previously.

The potted seedlings from the above experiments were kept in a greenhouse for five weeks and examined every two days. All experiments gave negative results.

An observation of note was made by Meijers (1938). Puccinia sorghi was unknown in Holland until a North American variety of maize was imported for experimental purposes. P. sorghi appeared on the first generation of this variety and subsequently on local varieties. Humphrey and Cromwell (1930) suggest that the introduction of Puccinia glumarum into Argentina may have been associated with the importation of grain. Naumov (1939), mentions that the possibility of inter-

continental spread of rust becomes more likely when one considers the many years of grain commerce between the continents, and that it only requires a single successful spore in the correct environment to establish the disease.

In the post war years there were large imports of maize into West Africa from the Americas, and especially into Ghana.

Introduction on a Living Host.

Records of grass importations kept by the Nigerian Federal Department of Agricultural Research revealed that clonal material of Tripsacum laxum, an alternate host of Puccinia polysora, was imported into Nigeria from Trinidad in 1945 and found to be infected with that rust. The clonal material was intercepted by the Plant Quarantine division and all infected plants destroyed. The remainder was sterilized and subsequently multiplied. Although a most careful watch was kept no further infection was observed. Subsequent cross inoculation studies have shown that T. laxum cannot be infected with the form of P. polysora on Zea mays in Nigeria.

Introduction of Inoculum by Air Transport.

Chester (1946) states that modern inter-continental air transport could be a most effective means of introducing rusts. The rapidity of travel would ensure that, if an aeroplane or its contents were contaminated, the uredospores would have an excellent chance of being liberated in a viable state at the point of arrival.

During the war and in the immediate post-war years, foodstuffs, including green vegetables, were flown into West Africa from the Americas to supply forces stationed on the coast. Among these fresh vegetables was sweet corn 'on the cob'. Uredosori of P. polysora have very frequently been observed growing on the inner surfaces of the husks of maize ears. Samples of maize grains, for experimental purposes, were also flown in frequently during the post-war years.

DISCUSSION

Most of the work on the limits of spore dispersal has been based on physical reasoning with no experimental backing. The very large number of spores expected to be released from the nearly infinite number of points at the origin of the rust, Central America and the West Indies, would have no absolute theoretical limit of dispersal. The chances are that spores often complete the 3,500 mile journey, but are almost certainly non-viable on arrival. Experiments made by the writer at Ibadan have shown that the uredospores of P. polysora failed to germinate after exposure to a temperature of 5°C for periods greater than 12 hours and at some stage of the journey over mid-Atlantic the spores would most certainly be subjected to such, and lower temperatures for long periods.

Present opinion tends to discount wind as a medium of dispersal of fungus spores over large areas of ocean and this is supported by present day species-isolation throughout the

world. Presumably P. polysora was in America for thousands of years prior to its discovery in 1897 and in all the years maize has been present in the African Continent, the rust has failed to establish itself there by natural processes.

Experiments on the inoculation of grain all gave negative results. These experiments, however, were not sufficiently comprehensive and, owing to the difficulty of simulating natural conditions of seed contamination experimentally, are far from being conclusive. The possibility of introduction by spores adhering to imported grain brought by a sea route would be greater in rusts in which the viability of the uredospore was longer. In laboratory tests at Ibadan P. polysora showed a reduction of viability of 50% after 27 days and total death after 50 days. The chances of survival during a long sea voyage are small, but the observations of Meijers (1938) and Humphrey and Cromwell (1930) indicate that the possibility of introductions by this method cannot be discounted completely.

It is unlikely that the rust was introduced on infected Tripsacum laxum in 1945. The remaining original material which was not destroyed was multiplied and distributed throughout Nigeria, and in the years between its introduction and the appearance of P. polysora on Zea mays, no rust was observed on the T. laxum, nor on Zea mays in Nigeria. The imported T. laxum was clonal material from Trinidad in which area it is commonly infected with a rust currently classified as P. polysora. Cross inoculation studies at Ibadan have repeatedly

failed to produce infection on T. laxum with inoculum obtained from infected Zea mays and it is possible that the respective strains (if both rusts are the same species) are incompatible on the two hosts. Unfortunately no similar cross inoculations have been done in Trinidad to support this hypothesis.

Introduction by air transport is likely. During the war and the immediate post-war years air traffic was considerable between the Americas and the West African Coast. Both green maize and dry grains were imported during the years immediately prior to the appearance of the disease. Spores adhering to maize grain, or in the aircraft, and liberated with dust and debris at the destination possibly would still be viable and could infect maize in the vicinity. Corn 'on the cob' was brought in regularly as a green vegetable and, if the husks were infected with rust, their discard with refuse would liberate uredospores from the sori on their inner surfaces.

Circumstantial evidence is indicative of introduction by air transport but this cannot be proven and the actual method of introduction of the disease may never be known.

SECTION 2.

Summary

1. The nomenclature and known host range of *P. polysora* are given.

2. The pattern of spread of *P. polysora* is described and the present known world distribution listed.

3. In the study of herbarium specimens of *P. polysora* from different areas of the world, variations in the dimensions of the uredospore were observed and analysed. It is suggested that geographical races of the rust may exist but no cross-inoculation studies have yet been done to confirm the existence of such races.

1. NOMENCLATURE OF THE PATHOGEN.

Puccinia polysora Underwood

Bull. Torrey Bot. Cl. 24 (1897) p.86.

Dicaeoma polysora Arthur

Res. Sci. Congr. Vienna (1906) p.344.

2. DESCRIPTION OF THE RUST.*

Uredosori	Pustules small and circular, sometimes slightly elongated, becoming densely and uniformly distributed, cinnamon to yellowish brown, commonly opening by a longitudinal slit.
Uredospores	Yellowish or golden, ellipsoid or oval, non pedicellate, 27 - 41 x 20 - 29μ, finely and sparsely echinulate, pores usually 5, equatorial.
Teleutosori	Sub-epidermal indehiscent, 0.5 - 1.5 mm. long, chocolate brown.
Teleutospores	Usually angular from pressure, pedicels persistent, brown, 29-41 x 19-27μ, wall 1.5μ, usually not much thickened above, pedicel short, mesospores common.

*This description has been approved by the Commonwealth Mycological Institute.

The aecidial and pycnidial stages are unknown in the areas of the world affected by P. polysora.

3. HOSTS.

Zea mays, Tripsacum laxum, T. latifolium, T. lanceolatum, Erianthus divaricatus, Euchlaena mexicana.

4. THE WORLD DISTRIBUTION OF PUCCINIA POLYSORA UNDERW.

Until the appearance of the rust disease of maize caused by Puccinia polysora in West Africa in 1949, knowledge of its world distribution was confined to reports from Central and South America and the West Indies. The first record was established by Underwood (1897) who described it as a new rust species on Tripsacum laxum in Alabama. Arthur (1920) recorded it on T. laxum in Florida, New Jersey and San Domingo and on other species of Tripsacum in Mexico and Cuba. Cummins (1941) identified it on a specimen of Zea mays from Peru, the first record of the rust on this host, and later found it to be of general occurrence on maize in Central and South America.

i) Central and South America, West Indies.

In 1945 the rust was observed on Zea mays in Trinidad and in 1947 in British Honduras. The following year, 1948, it was recorded in Jamaica. Information concerning the severity of

the disease in these areas is almost totally lacking, but it was reported that 'negligible' damage was caused in the West Indies.

ii) South-East Asia and Adjacent Islands.

Specimens of maize rust were collected in Malaya in 1950 and identified as P. polysora. In 1952 maize in British North Borneo was affected and, in 1956, in Siam, the Philippines and Christmas Island (Indian Ocean). Again reports of damage are scanty but in some of the affected areas the attack was widespread and severe in parts.

iii) The African Continent.

Deighton (1949) reported that maize rust during the 1949 growing season was widespread and severe in Sierra Leone. The rust was at first thought to be Puccinia sorghi Schw., previously recorded only once in Sierra Leone in 1943 (with teleutospores), but the virulence of the disease aroused suspicions. A critical examination was made by G.R. Bisby of Sierra Leone specimens collected in 1949 and of collections made in 1951 in Ghana and Nigeria, as a result of which he concluded that they belonged to P. polysora. G.B. Cummins confirmed these identifications after critical comparison with American specimens of P. polysora. After further examinations of all maize rust specimens from the African Continent by the Commonwealth Mycological Institute it was

concluded, with reasonable certainty, that P. polysora did not exist in Africa prior to 1949.

In 1950 the Department of Agriculture, Ghana, reported a 'generally distributed' outbreak of maize rust in that territory and it was observed that the highest incidence of rust was in the coastal rain forest areas, where rainfall and humidity are greatest, and that it was less severe in the drier areas of the interior. All specimens collected in Ghana during 1950 were identified as P. polysora. In mid-1950 the Departments of Agriculture of the French territories of Ivory Coast and Dahomey reported outbreaks of rust and mentioned that rust was most severe on maize planted late in the season and that early plantings escaped to an extent. In late 1950 the rust appeared in the south-west corner of Nigeria (Rhind, Waterson and Deighton, 1952). The following year, 1951, the rust was widespread throughout the Western and Eastern regions of Nigeria and had appeared in Cameroons under British Trusteeship. Nattrass (1952) reported P. polysora in Kenya in 1952. The previous year, the Kenya Government having been warned of the advent of P. polysora in West Africa, an examination of maize rust in the colony had revealed only P. sorghi. Also in 1952 it was reported in the Belgian Congo, Tanganyika and the Sudan. Early in 1953 the rust appeared in Nyasaland and Southern Rhodesia and later the same year in Madagascar, Mauritius and Reunion in the South Indian Ocean. In 1955 further reports were received from the Islands of Agalega and Rodriguez in the Indian Ocean.

The present known world distribution of P. polysora is given in Table 4 and in each case where a type specimen is retained by the Commonwealth Mycological Institute a Herbarium reference number is given.

Table 4.

The world distribution of *Puccinia polysora* (1957).
Dates of first appearance are listed in chronological order.

Territory	Date First Reported	C.M.I. Index No.	Host.
Alabama	1897	Det. Underwood	*Tripsacum laxum*
Florida	1920	Det. Arthur	*Tripsacum laxum*, *T. latifolium*.
Peru	1941	Det. Cummins	*Zea mays*
Trinidad	1945	1944	" "
Honduras	1947	47428	" "
Jamaica	1948	31440	" "
Sierra Leone	1949	38678	" "
Ivory Coast	1950	48685	" "
Ghana	1950	45931	" "
Dahomey	1950	47944	" "
Nigeria	1950	45926	" "
Malaya	1950	62164	" "
French Guinea	1951	53057	" "
Belgian Congo	1952	52565	" "
Sudan	1952	51198	" "
Kenya	1952	50121	" "
Tanganyika	1952	50581	" "
North Borneo	1952	62556	" "
Nyasaland	1953	52590	" "
N. Rhodesia	1953	52489	" "
S. Rhodesia	1953	56016	" "
Portuguese E. Africa	1953	Det. Carvalho	" "
Madagascar	1953	?	" "
Mauritius	1953	51927	" "
Reunion	1953	52909	" "
Agalega Island	1955	60590	" "
Rodriguez Island	1955	60203	" "
Christmas Island (Indian Ocean)	1956	62093	" "
Siam	1956	Det. Cummins	" "
Philippines	1956	Det. Cummins	" "

5. AN ANALYSIS OF THE SIZE FREQUENCIES OF UREDOSPORES OF GEOGRAPHICALLY SEPARATED POPULATIONS.

In order to determine whether or not uniformity existed in the dimensions of uredospores an analysis was made of collections from different areas in West Africa. Samples of a hundred spores from dried herbarium specimens from each of the following locations were measured. All samples were measured in glass-distilled water and in every case measurements were completed by one hour after mounting.

Territory	Number of Samples.
Sierra Leone	3
Ivory Coast	2
Ghana	11
Dahomey	2
Nigeria	14
British Cameroons	3

Frequency distribution curves were constructed from 500 measurements of lengths and breadths of uredospores collected at Ibadan, Nigeria during 1953 and all other West African samples listed above were subsequently compared with these curves. It was found that none departed significantly from the size distribution of the Ibadan sample. An analysis of uredospore measurements of West African ~~species~~ specimens is given in Table 5.

In 1956 F.C. Deighton, Commonwealth Mycological Institute, sent the writer specimens of maize rust Puccinia polysora, from

S.E. Asia and adjacent islands and mentioned that uredospores from that area were apparently smaller than in collections from Africa. Six samples, each of a hundred spores, were measured from Malayan material and two from Christmas Island (Indian Ocean). The analysis of these samples is also given in Table 5, and the results show a highly significant difference (P = 0.1%) both in lengths and breadths between West African and Malayan and Christmas Island spores.

Table 5.

An analysis of the measurements of uredospores of P. polysora from collections made in West Africa and in S.E. Asia and adjacent islands.

	West Africa	Malaya, Philippines, Christmas Island.
Mean length	32.70 ± 0.14 μ	28.67 ± 0.23 μ
Mean breadth	24.79 ± 0.24 μ	22.57 ± 0.31 μ
Correlation Coefficient of Lengths to Breadths	-0.28	-0.31
*Relative Volumes of Spores	1.0	0.78

Mean difference of Lengths	4.03 ± 0.38 μ
Mean difference of Breadths	2.20 ± 0.24 μ

*(Smith, 1953)

With the assistance of the Commonwealth Mycological Institute and the Arthur Herbarium, duplicates of all available specimens of P. polysora on Zea mays and on the alternate hosts

Tripsacum laxum and Euchlaena mexicana were obtained. Samples of 100 spores from a total of 65 specimens, representative of all areas of the world affected by the rust, were measured.

Results

Uredospores from herbarium specimens which have been identified as P. polysora fall on a size basis into two main groups. This grouping is illustrated in Text Fig. I. in which the mean size of samples is plotted. Samples obtained from different hosts are indicated by different symbols. This figure shows that there are two main forms of P. polysora on Zea mays when distinguished on a size basis. The smaller of those is centred on the S.E. Asia area and is consistent with the eastward spread of the rust from its first recorded appearance in S.E. Asia, i.e. from Malaya. Presumably the origin of the rust was once more America. Records from Borneo, however, are not consistent with this, the spores being much larger as shown in Figs. I and II. The larger spore form is found in the Caribbean area, Africa and islands in the South Indian Ocean and is consistent with the observed eastward spread of the rust shown in Table 5. Fig. II illustrates the variation in morphology with location. The histograms were each constructed from 200 measurements from herbarium samples of rust chosen at random from the areas mentioned. The mean size of each sample is marked by a vertical line.

DISCUSSION.

Data is lacking on cross-inoculation studies of cultures of P. polysora from areas outside Africa and it is not possible at present to determine whether or not biotypes exist. Attempts to infect Tripsacum laxum at Ibadan with West African uredospore material obtained from Zea mays have failed. The Tripsacum used was clonal material from the West Indies, in which area natural infection of this host by P. polysora occurs readily.

However it has not been established whether the forms of P. polysora on Zea mays and Tripsacum in the West Indies are identical. Further investigations are required to determine this point, using cross-inoculation methods and host material of known genetic identity. Until these inoculations have been done there is no justification for further assumptions on the relationships of the groupings in Fig.I. Whereas the Malaya, Christmas Island and Philippines group is significantly distinct from the African and West Indies group, the dimensions of uredospores from Central America and North Borneo do not fit into either of the two main groups.

The effect of environment on uredospore formation may be a contributory factor to the variations in spore size that have been found. Levine (1923) and Stakman and Levine (1919) have shown that environmental factors influence uredospore size in Puccinia graminis and Bailey (1925) observed that environmental conditions result in changes in uredospore size as great as, or even greater than, the differences between spores of different

races of Puccinia graminis avenae produced under similar conditions. Hingorani (1952) showed that significant fluctuations in uredospore size of races 2, 7 and 8 of Puccinia graminis avenae were induced by temperature, light intensity and the degree of resistance of the host. This last factor, especially, may be applicable to P. polysora but has not been investigated.

Levine (1923), Waterhouse (1930) and Manners (1950) have shown uredospore size variation in different physiologic races of Puccinia graminis and Puccinia glumarum respectively.

SECTION 3.

Summary

1. The symptoms of the disease on Zea mays L. in West Africa are described.

2. The life cycle of P. polysora is apparently reduced to the uredo- and teleuto- stages and the aecidial and pycnidial stages are unknown. All attempts to germinate teleutospores have failed. It is suggested that the life cycle has been conditioned by environment and reduced to an autoecious semi-form.

3. The effects of temperature and humidity on the germination of uredospores have been superficially investigated and it has been found that the prevailing climatic conditions on the West African coast provide near optimum conditions for uredospore germination.

4. The viability of the uredospore has not been found to exceed 50 days, either in controlled laboratory conditions or under natural conditions in the field. The rust perpetuates by chain infections in the uredo-stage.

5. Infection studies have shown that single uredospores can initiate infection. The percentage of successful infections obtained with single spore inoculations was greater in rust susceptible varieties than in resistant varieties.

1. THE SYMPTOMS OF THE DISEASE ON ZEA MAYS L.

The uredo- and teleutosori are found predominantly on the upper, and, to a lesser extent, on the lower, surface of the leaf, the husks of the ear, and rarely, on the most susceptible varieties, on the true stem at the nodes.

Uredosori occur on both sides of the leaf. Each pustule is formed from an individual infection and the mycelium is restricted to the host tissue immediately beneath the pustule. The uredosori are scattered at random over the entire surface of the leaf, are confined to the inter-vascular areas and, in severe infections, the pustule density may be as great as 50 per cm.sq. leaf area and the entire plant may assume a bright orange appearance. The uredosori are small and generally circular, sometimes oval, 0.2 - 1.3 mm. and never confluent.

When the uredosori are few and widely dispersed it is frequently seen that nine to ten days after the appearance of a pustule it becomes surrounded by a circle of secondary sori. A similar observation has been made with Puccinia graminis tritici by Arthur (1929), Allen (1926) and Dodov (1931). Histological examinations of these infections show no spread of the original mycelium and apparently the secondary sori are initiated by infections from the primary sorus.

In susceptible varieties the appearance of a green island, caused by the retention of chlorophyll in the senescent host

tissue, is evident around the pustules and is retained for a considerable period after the death of the surrounding leaf tissue. This symptom has been described in other rusts by von Tubeuf (1897) and D'Oliviera (1939, 1940).

The teleutosori are extremely rare in occurrence in West Africa. The sori are generally slightly elongated, 0.5 - 1.5 mm. long, and are chocolate brown in colour. They do not dehisce, remaining covered by the epidermis of the host and are difficult to detect. They occur on both surfaces of the leaf and are found at the senescence of the host plant.

2. THE LIFE CYCLE OF PUCCINIA POLYSORA.

Six days after the inoculation of a leaf surface with uredospores a faint, greenish-yellow spot, approximately 1mm. in diameter, appears at the infection site. The spot gradually yellows towards the centre and begins to distend on the seventh day after inoculation. On the eighth or ninth day the epidermis ruptures, generally by a longitudinal slit, revealing the mature uredospores which are immediately infective.

The teleuto-stage is very rare in occurrence. In December, 1953, teleutosori were first found on maize growing at Ibadan. In December the second annual crop is maturing and at this time the dry season is starting and the senescent maize crops are subjected to the Harmattan, a cold dry wind from the Sahara

causing very low humidity during the day and cold dry nights. It is only at this time of the year that teleutosori are formed and have been collected at that period in two successive years. In the montane areas of British Cameroons teleutosori are frequently found on maize growing at an altitude of 4000 ft., and Dr. H.H. Storey has also recorded a similar frequency of occurrence in the highlands of Kenya.

The aecidial and pycnidial stages of P. polysora are unknown. During the course of observations on the rust a constant watch has been kept on associates of Zea mays in the field but no related aecidia or pycnidia have been found, and in the American Continent, the centre of origin of the rust, these stages are also unknown.

2.1. Experiments in Attempt to Induce Germination of Teleutospores.

On the discovery of teleutospores in December 1953, preliminary experiments were carried out in an attempt to induce germination and determine the importance of the teleutostage in the life cycle of the rust. All experiments were unsuccessful and H.H. Storey, East African Agricultural and Forestry Research Organisation, Kenya, informed the writer that tests in Kenya have also been unsuccessful. A further series of experiments was designed at Ibadan and teleutospores subjected to various chemical and physical stimuli. Since all tests gave negative results they are only briefly described

below and summarised in Table 6.

a) Maneval (1922) obtained germination of teleutospores by alternately wetting and drying host tissue bearing sori and then floating on water for long periods. This was done with material of P. polysora with and without prior freezing. The various combinations of wetting and drying periods and testing times are shown in Table 6.

b) Johnson (1931) attempted to break the dormancy of teleutospores of Puccinia graminis tritici by freezing in a refrigerator for 2 - 10 days, spraying with water at 5°C for a week and then wetting and drying alternately for various times. A range of cooling times from five to twenty days at a temperature of +5°C was tried with P. polysora, followed by wetting and drying for different periods and finally floating the material on distilled water.

c) The method of Thiel and Weiss (1920) was tried both on fresh material and on material stored in the refrigerator at 10°C for various lengths of time. Teleutospores were exposed to chloroform vapour in a closed jar for a period of one minute and then immersed in 1% citric acid for fifteen minutes.

d) Fresh and previously-frozen material was immersed in acetic acid at dilutions of 0.1, 0.01 and 0.001 N. for periods ranging from 15 minutes to 5 hours.

e) Sulphuric acid at the same dilutions was tried and material immersed for periods ranging from 5 minutes to 24 hours.

f) Several lipoid solvents, acetone, ether, chloroform and carbon bisulphide were employed in an attempt to increase the permeability of the spore wall to water and oxygen. Infected material was immersed in the undiluted solvents for periods of 5 to 30 minutes, then washed in running water and floated on distilled water for 10 days.

Table 6.

Experiments to induce germination of teleutospores of *Puccinia polysora*.

Time of collection of material and the initial treatment.	Additional treatment prior to germination test.	Germination	Time of germination tests after collection of material.
1. Collected December, 1953 and stored in refrigerator at 10°C.	a. Stored at +5°C for 5,10,20 days, then wetted and dried alternately for two day periods 5,10,20 times.	Nil.	3, 6 and 12 months
	b. Frozen at -5°C for 5, 10, 20 days, wetted and dried ten times and floated on distilled water for ten days.	Nil.	3, 6 and 12 months
	c. Wetted and dried for 5,10,20 times and then floated on distilled water for 10 days.	Nil.	3, 6 and 12 months
	d. No further treatment Floated on distilled water for 10 days.	Nil.	3, 6 and 12 months
2. Collected December, 1953 and stored in sealed tins in the field.	Treatments as in 1a, b, c and d.	Nil.	3, 6 and 12 months
3. Collected in December, 1953 and stored in the laboratory at 27°C and 50% R.H.	Treatments as in 1a, b, c and d.	Nil.	3, 6 and 12 months

Table 6 (cont.)

Time of collection of material and the initial treatment.	Additional treatment prior to germination test.	Germination	Time of germination test after collection of material.
4. Collected December 1954 and stored in refrigerator at 10°C.	a. Material exposed to chloroform vapour for 1 minute and then immersed in 1% citric acid for 15 minutes.	Nil.	3, 6 and 12 months
	b. Material immersed in acetic acid, N 0.1, N 0.01, N 0.001, for periods of 5 minutes to 4 hours.	Nil.	3, 6 and 12 months.
	c. Material immersed in sulphuric acid, N 0.1, N 0.01, N 0.001, for periods of 5 minutes to 24 hours.	Nil.	3, 6 and 12 months
	d. Lipoid solvents. Material immersed in undiluted acetone, ether, chloroform and carbon bisulphide for periods of 5 minutes to 30 minutes.	Nil.	3, 6 and 12 months
	Treatments 4a,b,c and d were all washed in running water for one hour after immersion and then floated in distilled water for 10 days.		
5. Collected December 1954 and stored in the field in sealed tins.	Treatments as in 4a, b, c and d, followed by alternate wetting and drying, 5, 10 and 20 times prior to floating on distilled water.		
	b. Treatments as in 4a, b c and d with no further treatment.		

2.2. Discussion

On consideration of available evidence it is likely that Puccinia polysora is a micro-cyclic rust, the pycnidial and aecidial (0 and I) stages being absent. This, however, must only be a hypothesis at present since extensive inoculation studies have not been done, either in the presumed area of origin of the rust, the Americas, or in any of the territories now affected by the rust. The theory that the rust is a micro-cyclic form is strengthened by the failure to germinate teleutospores under experimental conditions in the laboratory. Again, however, the physical and chemical methods employed were far from comprehensive and the teleutospore may germinate when subjected to the correct stimulus or series of stimuli. It is possible that in the course of evolution of the host-parasite relationship that the pathogen has become separated from its aecidial host and that the teleutospore has in consequence lost the capacity to germinate. On examination of the nucleus after each of the tests listed in Table 6, it was observed to have remained in the expanded 'resting' phase (Savile 1939) and unchanged in appearance from the untreated spore. In fresh, untreated spores the nucleus appeared degenerate and dispersed but sufficiant work has not been done to prove the suggestion to be made that the teleutospore is a relic stage in the life cycle.

The rust is highly successful in the uredo-stage and,

as is more fully discussed in Sections 3 and 5, the ease of continuity in the uredo-stage in its present environment may have resulted in the suppression of the sexual phase in the life cycle.

In the absence of further information on the life cycle Puccinia polysora may provisionally be classified as a micro-cyclic, autoecious Hemiform.

3. THE UREDOSPORE

3.1. The Choice of Inoculum in Germination Studies.

Preliminary experiments on the effect of environment on the germination of uredospores were carried out with spores collected in the field by scraping them off the surface of infected leaves with a scalpel. Inconsistent germination percentages were obtained when several experiments were repeated under identical conditions. Schnaffnit (1909) contended that high germination percentages in vitro depended on the full maturation of the uredospore sample and that this maturation must be completed before natural detachment of uredospores would occur. Schall (1925) obtained higher germination percentages with uredospores of Puccinia triticina which had been dusted off than with those which had been scraped off. Rust infected leaves of Zea mays were collected in the field. Two series of petri dishes containing a thin, 1 mm., layer of non-nutrient agar were inoculated with uredospores, one series by tapping

infected leaves over the surface and the other by applying spores, previously scraped off the leaf with a scalpel, to the surface of the agar with a soft brush. The following results were obtained.

Method	Percentage Germination
(a) Spores tapped off infected leaves onto agar.	89-95%
(b) Spores scraped off with scalpel and brushed onto agar.	61-74%

Contributing to the lower percentage germination in method (b) may have been mechanical damage to a proportion of the spores by scraping but a more likely cause is the removal of immature spores by this method.

Spores collected in the field before and after rain gave different germination percentages. Schnaffnit (1909) found no viable uredospores after rain. Uredospores of P. polysora were collected from the surface of infected leaves in the field before and after rain with a cyclone spore trap (C.C.V. Batts, National Institute of Agricultural Engineering, Cambridge, Unpub.), a convenient method of collecting spores in bulk. The results of collections from two rust-susceptible varieties of Zea mays are given below.

Date	Variety	Percentage Germination Before Rain	: After Rain	Intensity of Rainfall
17.5.54	Lagos White	87	43	0.21"/73 mins.
	White Tuxpan	91	39	
19.5.54	Lagos White	91	26	0.93"/57 mins.
	White Tuxpan	93	23	

The large drop in germination was attributed to the removal of a proportion of the mature uredospores by the mechanical action of rain.

In order to ensure a uniform source of uredospores for germination studies, the collection of rust from the field was discontinued and rust was cultured on seedlings in the greenhouse. When required, infected leaves were detached and taken to the laboratory and the test plates inoculated by tapping the leaves over the surface of the agar. This method gave consistently uniform germination percentages.

3.2. The Effect of Temperature on Uredospore Germination.

Method.

Uredospores were collected daily as required from rusted seedlings grown in the greenhouse. A thin, approximately 0.5mm., layer of 3% non-nutrient agar was poured into the bottom of petri dishes and the under side of the lids covered with discs of moistened filter paper. Spores were applied to the agar by gently tapping infected leaves over the surface. For each test five plates were inoculated in this way and then placed in a cooling incubator (range 5 - 40°C.) at a fixed temperature and saturated humidity for 24 hours. Counts were made directly through the bottom of the petri dishes and ten low power fields chosen at random and the percentage germination determined.

Results.

This method gave consistent results on repetition of the experiment. Text Fig. III gives the germination curve obtained by this method and shows the high optimum temperature of 27°C. At the optimum temperature germ tube growth was vigorous, often reaching 800μ, often branching, with dense protoplasmic contents. At 10°C the germ tubes were absent or rudimentary, rarely exceeding 20μ, and appeared to have no contents. At 32°C the germ tubes were 10 - 50μ, vacuolated and often ruptured.

3.3. The Effect of Humidity on Uredospore Germination.

Literature relating to the effect of humidity on the germination of rust uredospores is extensive, especially concerning the wheat rusts, and all workers are agreed that saturation, or near-saturation is a requisite of germination. Mains (1924) stated that 100% R.H. is necessary for the germination of the uredospores of wheat leaf rust. Stock (1931) obtained low percentage germination oven at near saturation values unless the spores were actually in contact with water. Hemmi and Abbe (1933) obtained nearly total germination at saturated humidity and a very low percentage at 99% R.H. but maintained that the spores did not have to be in contact with the surface of water.

Method.

Microscope slides were dipped in molten 1% gelatine and the surplus drained off while still warm. Humidity chambers

were constructed from 10 oz. screw-capped jars. The controlling medium was placed in the bottom of the jar to a depth of 1" and the slide supported on two glass blocks ½" above the surface.

Difficulty was experienced in finding humidity controllers to operate at the optimum germination temperature of 27°C. The Physical Laboratories, Cambridge (in correspondence) gave the writer information on accurate control between 90 and 100% R.H. at a temperature of 25°C using super-saturate salt solutions and all results obtained refer to that temperature.

Uredospores were obtained from seedlings in the greenhouse and the surface of the gelatine on the slides was dusted with uredospores prior to being placed in the humidity chambers. Five replicates were made at each controlled humidity value and after insertion of the slides into the jars the screw-caps were tightly replaced and the jars placed in an incubator at a constant temperature of 25°C for 24 hours. The slides were removed and the percentage germination determined from the average of counts made in 10 low power fields of the microscope selected at random.

Results

Three series of tests gave the following germination percentages.

Table 7./

Table 7.

Percentage germination of uredospores of P. polysora at various relative humidities and a constant temperature of 25°C.

Relative Humidity (25°C)	Percentage Germination
100.00	90 - 95
98.00	80 - 87
97.45	0 - [illegible] (?)
92.48	0

Apparatus was not available for a more critical study of germination in relation to humidity but the experiment did indicate that the uredospores conformed with those of other rusts in requiring near saturation for germination.

3.4. The Viability of the Uredospore.

a) Viability of the uredospore when stored in the laboratory at a constant temperature and various relative humidities.

A series of humidity chambers was constructed from screw-capped jars as previously described and dilutions of sulphuric acid placed in the jars to a depth of 1", giving a range of constant humidities from 10 to 100% R.H. at 25°C. Uredospores were collected from seedlings in the greenhouse with a cyclone spore-trap and approximately 0.1 gm. was placed in each jar, spread thinly over the surface of a filter paper suspended 1" above the level of the acid. The jars were placed in an

incubator at a temperature of 25°C and a sample of spores removed from each jar every two days and the percentage germination determined on 3% agar plates at 27°C and saturated humidity. The absolute viabilities of the samples stored at the different humidities are given below.

Table 8.

The viability of uredospores of P. polysora stored at 25°C and various constant relative humidities.

Relative Humidity % (25°C)	Viability in days
10	18
25	16
35	18
50	48
65	62
75	60
90	32
100	Mould developed in two days. No germination counts possible.

Very low and near-saturate humidities evidently decrease viability, an observation also made by Raeder and Bever (1931) with Puccinia glumarum. It proved impossible to determine the viability at 100% R.H. owing to the rapid development of moulds on the spores, but from results obtained in the experiments on the effect of temperature on germination it is likely that the spores germinated immediately.

b) The viability of attached and detached uredospores stored in the laboratory at a constant temperature and humidity.

Two series of five humidity chambers were constructed, one containing small samples of uredospores collected with the cyclone spore-trap, and the other containing small pieces of maize leaves bearing uredosori, each piece measuring approximately 1 x 1 cm. The humidity in the chambers was controlled by a super-saturated solution of calcium nitrate which maintains a value of 51% R.H. at a temperature of 24.5°C. The two series of jars were placed in an incubator at that temperature, a sample of spores being removed from each jar each week and the percentage germination determined. The viabilities obtained from material stored under these conditions are given in Table 9.

The spores used in the first experiment were collected from a highly susceptible variety of maize, Lagos White. The experiment was repeated using spores from a resistant variety, Mexico 5, and it was found that the viabilities of spores from this variety did not vary significantly from the values in Table 9, the detached uredospores in each case having a shorter viability than those still attached to the host tissue during the storage period.

Table 9./

Table 9.

The viability of attached and detached uredospores of P. polysora stored at a constant temperature of 24.5°C. and 51% R.H.

	Time in Weeks	Percentage Germination.
Attached Uredospores	1	88
	2	60
	3	54
	4	48
	5	32
	6	20
	7	7
	8	0
Detached Uredospores	1	80
	2	75
	3	43
	4	24
	5	10
	6	0

c) The viability of the uredospores stored under field conditions.

During 1953 collections of infected maize were made at monthly intervals throughout the year, the material being obtained from plots of the variety Lagos White planted at ten day intervals. Each month a small quantity of rusted maize

was stored in a wicker basket in an open shelter in the field. A germination test was made on each sample at five day intervals. Table 10 gives the absolute viability in days for each collection.

Table 10.

The seasonal variation in the viability of uredospores of P. polysora during 1953.

Month of collection of material	Viability in days (5 day intervals)	Mean Max. Temperature	Mean Min. Temperature	R.H.%	Rainfall (ins.)
January	50	92.5	72.2	49.6	0.15
February	50	90.7	71.9	50.4	3.67
March	50	91.6	72.3	58.6	2.49
April	40	92.5	72.2	65.2	3.47
May	45	88.7	71.6	75.4	8.59
June	45	85.2	70.6	83.8	6.85
July	30	82.6	70.8	83.8	3.50
August	30	81.1	69.4	78.0	1.00
September	30	83.9	71.0	74.5	6.83
October	30	86.2	70.7	78.6	6.00
November	35	88.5	70.4	62.3	2.36
December	50	90.3	65.2	45.5	0.21

Mean monthly climatic data have been incorporated in Table 10 to illustrate the changes in viability in relation to season. It is apparent that longer viability is associated with high day temperatures, and low humidity and rainfall.

During the months of the rains, when the reverse climatic conditions prevail, the viability is greatly shortened. In the laboratory it was also found that spores stored at high relative humidity have short viabilities. During the rains the dew period may be 12 hours, and often longer, in duration and it is possible that the subjection of uredospores to long periods of high humidity shortens their viability.

3.5. The Survival of the Rust During Unfavourable Periods.

For the rust to survive from one maize crop to the next it is necessary for it to endure two inter-crop periods each year. The general practice in the south of Nigeria is to plant the main crop in April and harvest in late June, and the second crop in early September and harvest it in November. Following the experiments in uredospore viability, in which the viability in the field was not found to exceed 50 days, the method of 'carry over' of the rust was investigated.

The continuity of infection from the first to second season crops presented no difficulty owing to the wide latitude of planting dates encountered in local husbandry methods, and direct infection of the second crop could occur from mature, late planted first season crops.

The infection of the early crop each year necessitated the rust surviving the dry months of November to April, during which maize is not normally grown, and this period is far in excess of the viability of uredospores as determined experiment-

ally. A search was made for alternative hosts and Tripsacum laxum, maintained in small experimental plots at various agricultural stations throughout Nigeria, was examined throughout the dry months but no infection found. Collections of plant rusts were made during the dry seasons of 1953-55 and 23 Puccinia spp. obtained, none of which proved, on examination, to be Puccinia polysora. It was determined, however, with an automatic volumetric spore-trap (Cammack, 1957. In press), that viable uredospores of P. polysora were present in the atmosphere throughout the dry months indicating that a source of inoculum capable of infecting the first season maize crop was present in the atmosphere when the crop reached a susceptible stage of growth.

During these observations the writer observed in the township of Ibadan that it was a common practice to grow a few stands of maize in watered plots in compounds during the dry season. It was later determined that in the south-west corner of Nigeria, and in the fresh water swamp communities on the coast, it was a local procedure to grow 'out of season' maize in small irrigated plots on the banks of streams etc., and these were often seen to be heavily rusted. While collecting information for the rust assay (Sect. 5.2) it was noticed that first reports of rust on first season maize came from this area each year and it is most likely that the rust is carried through the dry seasons in this way, infects the local first season crops, which are generally planted very early in March, and

eventually spreads northwards each year, the inoculum being carried on the prevailing south-west monsoon wind.

This method provides a simple way for the rust to survive the unfavourable dry season and would explain the presence of viable inoculum in the atmosphere throughout the year. 'Chain infection' was proved possible experimentally at Ibadan on adjacent plots of rust-susceptible maize planted at ten day intervals throughout the year. Natural infection occurred on every plot.

4. INFECTION STUDIES.

4.1. The Numerical Threshold of Infection

The numerical threshold, or the minimum number of infective spores required to ensure infection, has been studied in different pathogens by several workers, notably by Heald (1921), Brown (1922), Dickson (1923), Glynne (1925), Haymaker (1928) and Price (1930). It is generally accepted that the rusts belong to the group of fungal pathogens in which, under optimum conditions of infection, a numerical threshold of one spore is sufficient to institute infection, as opposed to other pathogens requiring a larger threshold number. Experiments are described below which were designed to determine the inoculum density required to establish infection with P. polysora on seedlings of resistant and susceptible varieties of Zea mays.

Materials and methods.

Seedlings of a rust susceptible variety, Lagos White, and a resistant, Mexico 13, were grown in soil blocks (May, 1954) in the greenhouse. The seedlings were grown within cages of treble layer fine mesh muslin to reduce the chances of natural infection prior to inoculation. Quantities of uredospores were collected on the day of inoculation from stock seedling cultures in the greenhouse and the spores removed by tapping the infected leaves over a sheet of paper; this method being preferable to other methods for the reasons stated in Sect. 3. 3.1. Uredospores were added to a quantity of 0.1% solution of non-nutrient agar in distilled water and carefully mixed with a glass rod. Water alone as a suspending medium was discarded owing to the spores clumping and floating on the surface. The viscid gelatine aided even distribution of spores in the suspension. After thorough mixing the spore concentration per unit volume was determined with a 'Thoma' blood cell counting-slide and the suspension diluted as necessary to give an estimated concentration of 5000 spores/ ml. The original suspension was intentionally made denser than required in order that the correct concentration might be obtained by dilution. After adjustment a check count was made with the 'Thoma' slide. On correction of the original suspension a further series of dilutions was made containing approximately 2500, 500 and 100 spores per ml.

When the seedlings had grown to the stage of emergence of the third leaf they were removed to the laboratory for inoculation. An 'Agla' micrometer syringe was used to inoculate the surface of the leaves, the seedling being placed under the syringe with the delivery needle touching the surface of the second leaf, approximately 1 cm. from the tip and 0.25 cm. from one margin of the leaf. This infection point was selected after the experiments on single spore inoculation described in Section 3. 4.2. A volume of 0.01 ml. of the appropriate spore dilution was carefully applied to the surface of the leaf to form an 'infection droplet' and the seedlings immediately covered with a polythene incubation hood supported on a wire frame. To ensure saturate humidity, the hood was sprayed with water on the inner surface with an atomiser prior to being placed in position. In the first experiment 20 seedlings of each variety were inoculated with each spore dilution. At the dilutions used 0.01 ml. in each case should have given theoretical applications of 50, 25, 10, 5 and 1 uredospores respectively. The incubation hoods were removed 48 hours after inoculation.

Results.

During the incubation phase some of the inoculated leaves died back from the tip and in some groups a few of the controls showed natural infections. Table 11 lists the infections obtained at the respective dilutions.

Table 11.

The numbers of infections obtained on seedlings of a resistant and a susceptible variety of Zea mays on inoculation with different concentrations of uredospores of P. polysora in suspension.

Theoretical No. of Spores Applied	No. of Seedlings Inoculated	No. of Seedlings Assessed	No. of Seedlings Infected	No. of controls infected (20 in each group)
Susceptible Variety, Lagos White.				
50	20	19	19(100%)	1
25	20	17	16(89%)	0
10	20	20	17(85%)	0
5	20	19	15(79%)	3
1	20	19	3(15%)	0
Resistant Variety, Mexico 13.				
50	20	20	19(100%)	0
25	20	18	17(94%)	2
10	20	19	9(47%)	1
5	20	19	3(15%)	0
1	20	20	1(5%)	0

On inspection of the percentage infections obtained with the various spore concentrations it was observed that with low levels of application (10, 5 and 1 spores) higher infection percentages were obtained in the rust susceptible variety than in the resistant. The percentage infections obtained are significantly different in the two varieties at the 5%

probability level. The differences between the percentage infection at the single spore application is not significant at the sample size employed.

The experiment was repeated using a larger sample number and more varieties. Seedlings of the varieties listed in Table 12 were grown in soil blocks and inoculated as previously at the tip of the second leaf. Fifty seedlings of each variety were inoculated with theoretical spore numbers of 25, 10, 5 and 1, giving a sample of 200 seedlings of each variety. The experiment was replicated twice so that for each variety at each spore concentration the sample number was 150 plants. Each series has a similar number of untreated controls. The results are given in Table 12 and expressed as the percentage of infection obtained with each concentration of inoculum.

The most significant differences of percentage infections were obtained with the intermediate spore concentrations, especially the applications of five spores. With the exception of the highly resistant variety Eafro 880 there were no significant differences in the infections obtained subsequent to the theoretical application of a single spore, as was also observed in the first experiment. With the exception of the same variety, high infection percentages were obtained with an application of 25 spores in both resistant and susceptible varieties.

The relative percentages obtained in these experiments follow a Poisson distribution, e^{-m}, which is to be expected

especially at low dilutions where some inoculations must fail to contain a spore or may contain non-viable spores.

Table 12.

The infection percentages obtained on seedlings of resistant and susceptible varieties of Zea mays on inoculation with different concentrations of uredospores of P. polysora in suspension.

Variety	Percentage Infection Obtained at Theoretical Spore Applications of 1	5	10	25
Susceptible				
Abakaliki Red Flour	8 ± 2.7	56 ± 4.9	73 ± 4.4	97 ± 1.7
Tsolo	2 ± 1.4	44 ± 4.9	63 ± 4.8	93 ± 2.5
American Bounty	3 ± 1.7	43 ± 4.9	84 ± 3.7	81 ± 3.9
Tall Western Dent	3 ± 1.7	58 ± 4.4	88 ± 3.2	92 ± 2.7
Resistant				
Mexico 13 (SLP20 4A)	0	17 ± 3.7	51 ± 4.9	71 ± 4.5
Mexico 5	2 ± 1.4	15 ± 3.6	46 ± 4.9	86 ± 3.4
Eafro 220	3 ± 1.7	17 ± 3.7	58 ± 4.9	85 ± 3.5
Eafro 880	0	4 ± 1.9	24 ± 4.2	49 ± 5.0

4.2. Single Spore Inoculations.

It is well known that any technique of spore dilution is subject to greatest error at low concentrations of spores per unit volume. On the theoretical application of single spores in the experiments described in Section 3.4.1. it is possible that the inoculation did not contain a spore or that it contained more than one, and also that aviata spores were present in

the suspension. The infection percentages obtained by inoculating one spore were similar in both susceptible and resistant varieties of maize and the differences were not significant. It was suspected that a large error was present in the results obtained by the spore dilution method used and a technique of single spore inoculation was devised, both to test the dilution method for error and as a preliminary method for strain isolation.

Methods.

Several techniques of single spore inoculation were tested, each incorporating a Cook Hydraulic Micro-manipulator. These are briefly described below.

i) The dry needle method.

A microscope slide was spirit cleaned and then coated on one side with warm 2% agar, the surplus drained off, and allowed to set. B.D.H. non-nutrient agar proved to be the most suitable. Rust infected leaves of maize seedlings were gently tapped over the surface of the slide in order that the liberated spores formed a thin, and uniformly distributed, layer over the surface of the agar. The slide was transferred to the microscope stage and the needle of the manipulator adjusted under low power magnification. Two types of dry needle were tried, each fashioned from platinum-iridium wire; the spatulate form described by Keitt (1915) and the 'Dutch hoe' employed by Lindegren (1932). The former proved the more simple to operate and the spore was removed by sliding the

spatulate tip of the needle into the agar film beneath the spore and lifting off. The spore adhered readily to the agar which in turn was easily lifted by the needle and then transferred to the tip of the seedling leaf laid on the stage of a binocular, long focus microscope. This method was eventually discarded because of the difficulty of transferring the spore from the needle to the surface of the leaf, due to the adhesion of the agar to the needle. Mechanical damage to the leaf surface often resulted and presumably, in many instances, to the spore also.

ii) The wet needle method.

A second method using a wetted needle, similar to that used by Afanasiev (1937) was used successfully. Uredospores were tapped onto the surface of a dry, spirit cleaned slide. A blunt tungsten needle dipped in a weak solution of 'Teepol' detergent in distilled water (1ml./1000 ml. water) was placed over the selected spore and lowered until it made contact. If the surface of the microscope slide was perfectly dry and clean, single spores could be lifted easily in this way. Prior to transfer to the leaf the inoculation site was lightly sprayed with water from an atomiser and the needle brought in contact with one of the 'infection droplets'. The spore was easily transferred from the needle and held on the surface of the droplet by surface tension. In many instances, however, the spore was lost during transfer by vibration of the needle and by air currents.

iii) The micropipette method.

Micropipettes were drawn by the method described by Gee and Hunt (1928) and mounted on the micromanipulator. The proximal end of the pipette had an inside diameter of approximately 100μ. The free end was connected by rubber capillary tubing to an 'Agla' micrometer syringe which, on adjustment, could be used to control the filling and evacuation of the pipette. The plunger of the syringe was spring loaded to the body to ensure that the head of the plunger was always in contact with the micrometer. A cavity slide was filled with glass-distilled water and spores tapped on to the surface, as before. Single spores were withdrawn in small quantities of water and transferred immediately to the leaf surface; the water forming an 'infection droplet'. Care had to be taken to ensure that other spores did not adhere to the outside of the tip of the capillary tube. This method proved the simplest to use when large samples of seedlings had to be inoculated.

Using Method (iii) a series of seedlings of varieties of Zea mays, covering the full range of susceptibility to P. polysora was inoculated. The seedlings were covered with incubation hoods for 48 hours, and assessed on the 9th day after inoculation. Each series consisted of 50 inoculations, replicated four times, with the exception of two varieties of maize in which seed was scarce, and each had the same number of untreated controls. A proportion of the inoculated seedlings was not assessed due to damage or death of the inoculated area

on the second leaf. The infection percentages on the several varieties are given in Table 13.

Table 13

The infection percentages obtained on seedlings of resistant, intermediate and susceptible varieties of Zea mays on single spore inoculations with uredospores of P. polysora.

Variety	Qualitative Rust Reaction Type	Number inoculated.	Infections/ Total Assessed	Percentage Infection
Mexico 13 (SLP20 4A)	0	127	3/124	2
Eafro 880	0	43	3/43	8
Eafro 53193	1	143	10/129	8
Mexico 5	1	250	15/219	7
Tsolo	2	250	12/247	5
BR 129 Haiti	2	250	15/218	7
Abakaliki Red Flour	4	250	49/236	21
Yellow Tuxpan - 12	4	250	26/231	11
Lagos White	4	250	56/242	23

In all cases the infection percentages obtained by the single spore inoculation technique were greater than those given for the same varieties in Table 12, using the spore dilution technique. The greater number of successful infections cannot be entirely explained by the large sample number used in Table 13. , and suggests that the spore dilution method is very inaccurate at high dilutions. The class 4 rust susceptible

varieties all gave a high percentage infection but the distinction between the resistant, class 1, and the intermediate class 2, was not so marked and not significant at the 5% legel. No significant difference was found between resistant and susceptible varieties using the dilution technique at the single spore application, which also indicated the inaccuracy of this method in the determination of the numerical threshold.

SECTION 4.

Summary

1. A varietal survey of maize lines imported from all areas of the world showed that varieties from Central and South America possessed resistance to Puccinia polysora. All West African varieties were highly susceptible.

2. Quantitative and qualitative rust assessment methods were devised and their uses described in mass selection in the field and in critical varietal studies in the greenhouse.

3. The soil block method of maize seedling culture is described. Local materials proved suitable in formulating a compost and healthy seedling growth, necessary for inoculation studies, was maintained for three weeks from germination without the addition of further nutrients to the compost.

4. A standard seedling inoculation technique is now in use for the critical evaluation of the rust reaction of maize seedlings.

5. A study of the adult and juvenile qualitative reactions of maize to the rust has shown that in general the seedling displays a higher order of resistance than that shown by the adult in the field.

1. INTRODUCTION: SOURCES OF TEST VARIETIES OF ZEA MAYS

As mentioned in Section 1, P. polysora was known to be confined to Central and South America prior to its appearance in West Africa in 1949. In America the rust was generally reported as 'slight' or 'traces' and never in epiphytotic proportions, but in West Africa the severity of the outbreak suggested that the rust had encountered a new land race of Zea mays which had never possessed or had lost the factors for resistance which were evidently present at the origin of the rust in America.

The most successful method of combating the cereal rusts is by introducing genes for resistance and for this purpose the West African Maize Research Unit collected a wide range of test lines from all maize growing areas of the world with special reference to Central and South America. In the latter part of 1951 requests were sent out by the Nigerian Department of Agriculture for supplies of maize varieties from North, Central and South America, the West Indies, East, Central and South Africa, India, Malaya and Australia. A full list of these acquisitions is given in Appendix 2 of the First Annual Report of the W.A. Maize Research Unit (1953). During 1952 more than 300 varieties were received and this large number necessitated a simple and rapid method of field assessment being devised in order that primary selections could be made for incorporation in the breeding programme. The varieties were sown in simple

replicated line blocks in the field, the lines being inter-spaced with 'spreader lines' of the rust susceptible variety, Lagos White, to ensure that the test lines were subjected to infection by abundant inoculum.

2. RUST ASSESSMENT OF THE ADULT HOST IN THE FIELD.

2.1. The Quantitative Rust Assessment Method.

In order that selections could be made from the large number of introduced maize varieties two main requirements were necessary in the quantitative assessment of rust in the field.

a) The assessment key must be simple and capable of being used by unskilled observers.

b) The assessment of rust must be made on leaves representative of the whole plant.

During the first season planting, 1953, observations on the onset and pattern of spread of rust were made on 500 individuals of two varieties of maize, planted on the 17th March. These were the local and rust susceptible Lagos White, and the introduced and resistant Mexico 1. Both had a maturity time of approximately 12 weeks allowing a direct comparison of the respective patterns of rust attack. The populations were observed every five days and leaf numbers recorded, and every two days for the time of onset and spread of rust. The results are given in Table 14 and summarised at 20 day intervals.

Table 14.

Observations on the incidence of rust on a susceptible and a resistant variety of Zea mays.

Growth Stage	Days after Germination	Mean Number of Leaves	Mean Proportion of Leaves Infected.
Lagos White (Susceptible)			
3rd leaf	10	2 - 3	0
5th-6th leaf	30	6	0
Tasseling	50	11	2/11
Milk Ripe	70	12	10/12
Maturity	85	7	7/7
Mexico 1 (Resistant)			
3rd leaf emergence	10	2 - 3	0
5th-7th leaf	30	7	0
Tasseling	50	12	3/12
Milk Ripe	70	14	9/14
Maturity	85-90	10	9/10

These observations gave information on the distribution of rust on the plant and on the leaves which could be taken as representative of the degree of infection present at any one time. Examination of the pattern of spread on the individual plants showed that invariably the onset of rust occurred on the oldest leaves at the base of the plant and spread towards the apex, infecting each leaf in series.

In order that continuity of observations on the development of the rust could be obtained it was necessary to determine which leaves were healthy throughout the period from onset of rust until maturity of the host and this was done by tag-labelling each leaf of 100 plants of each variety on emergence.

The results of the preliminary field observations prior to the construction of an assessment key are summarised below.

a) Assessment should only be done on leaves which are sufficiently mature to be receptive to rust at the time of assessment.

b) Only those leaves which remain green during the period from natural time of onset of rust until maturity of the host should be used. Only the 5th and subsequent leaves are suitable, those prior to the 5th dying early.

c) A convention must be adopted to ensure the correct numbering of leaves. This was done eventually by numbering, in sequence, the leaves above the second stem node above ground level (the second buttress) at the 30th day after germination.

d) Leaves which are representative of the degree of rust incidence on the whole plant should be assessed. The 5th, 7th and 9th leaves above the second stem node were chosen.

The quantitative assessment key./

The quantitative assessment key.

The assessment key finally employed was based on the field observations described. It was modified as required from the Cobb scale (1892) and has five degrees of rust intensity, 0 - 4, corresponding respectively to 0,1,10,50 and 100% leaf area affected by rust. The five categories are listed below.

Degree of rust intensity.		Appearance of host leaf.
Class	0	The host leaf green and healthy. Occasional chlorotic spots may be present.
Class	1 (1%)	Single isolated pustules scattered over the surface of the leaf. Never in clusters.
Class	2 (10%)	Small groups of pustules distributed over the surface and generally interspaced with occasional single pustules. The leaf still appears healthy, chlorosis being confined to areas immediately around the pustules.
Class	3 (50%)	Groups of pustules greatly enlarged and beginning to coalesce. The leaf has lost its vigour and there is marked general chlorosis over the infected areas.
Class	4. (100%)	Pustule distribution general and dense over the entire leaf surface. The leaf assumes a striking orange-red colouration and extensive chlorosis precedes the death of the leaf.

To simplify the use of the key by unskilled observers it is mounted on stiff board and arranged so that it slides past the upper of two observation windows, the lower of which is placed directly over the leaf being assessed. By sliding the

scale along its slot a direct matching of intensities can be obtained, whereupon the observer reads off the appropriate rust number. The assessment key is shown in Fig. IV, and in use in Fig. V.

In the field assessment was done on 100 individuals taken at random from each maize variety being tested. The 5th, 7th and 9th leaves above the second stem node were assessed at the mid-blade position on four dates; these dates being the 30th, 50th, 70th and 85th days after germination, corresponding to the 5th-6th leaf, tasseling, milk-ripe and maturity growth stages respectively.

2.2. The Distribution and Classification of Rust Resistance.

The sources of rust resistance found in the first variety survey are described by Stanton and Cammack (1953) and can be generally classified as

Source of varieties	Degree of resistance to P. polysora.
West Africa East Africa South Africa India Ceylon Malaya	No resistance found.
United States	Of variable resistance.
Mexico Caribbean Venezuela	Several tolerant and highly resistant varieties.

It was found that resistance within the 327 varieties tested ranged from that in which all the plants were classified as showing the highest proportion of Class 1 rust intensity, the rust resistant varieties, to that in which all the plants were classified as showing the highest proportion of intensity Class 4, the most rust susceptible. In between these two extremes were varieties of greater heterogeneity, having a proportion of the plant population in two or more of the assessment classes.

From an inspection of the results expressed as frequency histograms for each of the varieties it was found that these could all be placed in one or other of a total of nine types. Characteristic groups are illustrated by frequency histograms in Fig. VI. Class 0 has been omitted from the histograms, not having been found in the field.

An analysis of the results of all the varieties assessed for rust resistance is given in Table 15. The varieties are grouped according to their rust reaction and their country of origin. A brief description is given in the Table of the characteristics of each of the groups. Types 3a and 5a are so numbered because of their affinity to the mono-modal frequencies in group 3 and 5 respectively. It was evident in the early stages of the breeding programme that many of the varieties under test were heterogeneous, and that the representation of resistance by a single numerical value would lead to a false conclusion about the varieties. The method adopted provided a better picture of their rust reaction.

Table 15.

Results of tests for rust susceptibility showing maize varieties grouped according to host reaction.

RESULTS OF TESTS FOR RUST SUSCEPTIBILITY SHOWING MAIZE VARIETIES GROUPED ACCORDING TO HOST REACTION

(The name of the variety is followed by its acquisition number. See Appendix II) (Varieties in italics have been tested more than once.)

	GROUP A RESISTANT			GROUP B INTERMEDIATE			GROUP C SUSCEPTIBLE		
HOST REACTION (See Fig. 1) Country of Origin.	TYPE 1 More than 95% of the plants in the variety show resistance of the first degree.	TYPE 2 More than 75% but less than 95% of the plants show resistance of the first degree.	TYPE 3 Plants of the first degree of resistance are more frequent than plants of other degrees but constitute less than 75% of the total.	TYPE 3A Degrees of rust resistance Nos. 1 & 4 are more frequent than others. The frequency in a given population shows a bimodal distribution over the range.	TYPE 4 Plants of degree of resistance No. 2 are more frequent than others.	TYPE 5a Plants of degrees of resistance Nos. 2 and 4 are most frequent. Frequency shows a bimodal distribution over the range.	TYPE 5 Plants of degree of rust resistance No. 4 are more frequent than plants of other degrees but less than 75% of the total.	TYPE 6 More than 75% but less than 95% of the plants showing a degree of resistance No. 4.	TYPE 7 More than 95% of the plants showing a degree of resistance No. 4.
AMERICA CENTRAL AMERICA	*Mexican* 1 (83) Mexican 5 (87) Mexican 17 (99)	*Mexican* 3 (85) *Mexican* 7 (89) *Mexican* 14 (96) *Mexican* 16 (98) *Mexican* 20 (102) Mexican 21 (103) Mexican 22 (104)	*Cuba Amarillo* (117) La Creole Y.F. (Bot.) Mexican 4 (86) *Sicaragua* 40 Venezuela 1 (37)			*Mexican* 15 (97) *Mexican* 18 (100)			
U.S.A.		*N.C.* 1032 (18)		C. 50 (Bot.) Jellicourse (8) *Whatley Prolific* (9)	*White Tuxpan* (10)				N.C. 27 (17)
AFRICA SIERRA LEONE		Mende (166)						Sierra Leone 1 (77) Hoorefiej (78) Marke (79) Sierra Leone 4 (80) Bakama (81) Sumbala (82)	Aku (164) Bulom
GOLD COAST					Sunyani (174)		Wenchi (173)	*Mankessim*	*Akwanga* and others *Bunsu* and others Kumasi (175)
NIGERIA									*Lagos White, Abakaliki White flour, Ibadan White flour, Ilorin White flour* and others
SOUTH AFRICA		Big Joe (14)	Tsolo (16) *Tsolo synthetic* (Bot.)				American White Flint (3)	Natal 8 Row (5) Dixie 18 (15)	Wisconsin White Dent (2) Cincinatti Bushman (4)
EAST AFRICA		*Kitale Flat White* (50) African grown maize (55)	Kenya (70) Kitale (76)		*Hickory King* (59) Kenya (66)	Common White maize (53) Kenya flat Yellow (65) Kenya Hybrid (68)	*Natal Horse Tooth* (56) *Kenya* (68) *Muratha* (52) *Mauritius* (136)	*Kenya* (64) *Korontari* (108)	
ASIA INDIA						*Kampar* (143) *White Velda* (124) *Red Velda* (125)	Pusa Yellow (144) *Arwhari* (127) *Duahmoger* (122) *Sarawah* (126)		
CEYLON					*Wellawaya* (129)		*Batticolea* (133) *Embilliptuya* (131) Maba-Oya (128) Pelwehera (138)	Hill (139)	
MALAYA						Malaya Red Flint (106)	Malaya Yellow Flint (107)		
AUSTRALIA									Improved Yellow Dent (48)

2.3. Discussion

When the preliminary results of field selection for resistance to rust were circulated, the use of an assessment key based on pustule frequency was severely criticised by several workers. The method was introduced in the early stages of field selection purely as an interim measure. It has, however, been retained for mass selection in the field since its extensive use has substantiated its worth as a convenient method of primary selection. Field experiments have shown that known applications of uredospores, sprayed on in suspension, result in a higher frequency of pustule formation on susceptible maize varieties than on resistants. Also, experiments described in Section 3.4.1 showed that identical applications of inoculum resulted in a higher proportion of subsequent infections on susceptible than on resistant varieties.

It is maintained that in field selection where all individuals of all varieties are subjected to the same concentration of airborne inoculum and the same conditions of infection, the resulting frequency of formation of pustules is a measure of the susceptibility of the individual or variety. When a qualitative assessment was devised it was found that the distribution of resistance determined by this method was broadly the same as that found in the field using the quantitative key.

With reference to the results obtained in the field survey, only seven varieties were found in which the frequency of rust intensity reaction was normally distributed over the range

(Fig. VI, type 4.). The majority are either classifiable as clearly resistant or susceptible.

The main feature of these results is, therefore, that the varieties may be classified geographically into 'New World' varieties, bearing resistance to P. polysora, and 'Old World' varieties, (Africa and Asia), in which resistance is absent. The distinction is not as marked as in natural populations, geographically isolated, since there have been recent importations of varieties from U.S.A. to parts of Africa, especially South Africa. This has tended to restore the identity between the two groups.

3. JUVENILE ASSESSMENT IN THE GREENHOUSE.

3.1. The Greenhouse.

The function of a greenhouse in the tropics is superficially the same as that of a greenhouse in colder climates in that both are designed to control the environment. Here, however, the similarity ends in that in the tropics the house is designed to control

a) Humidity

b) The biotic environment (insects and fungal diseases)

c) Excess insolation.

The relative importance of these factors depends on the particular studies being undertaken. The main problem

encountered in growing maize seedlings was that of maintaining high light intensity without excessive rise in temperature. Excessive insolation causes high temperatures during the day and if shading is employed to control day temperatures, to be effective it has to be taken to a degree which is detrimental to plant growth. In the early stages of experimentation adequate temperature control was obtained by heavy shading but weak, etiolated seedlings resulted which were totally unsuitable for infection studies. The difficulty was finally overcome by installing a powerful extractor fan on the gable immediately under the ridge. This kept the layer of air directly under the roof glass in motion, acting as a heat extractor and allowing a much greater area of glass to be used without a consequent rise in temperature. The greenhouse at present in use has the following dimensions:-

Height 2.75 m. to ridge, 2.14 m. to eaves.

Base 3.05 x 9.15 m. (vol. 84.8 cu.m.)

Roof $\frac{2}{3}$ glass plate, $\frac{1}{3}$ wood boarding.

Walls Up to 1 m. brick. Copper gauze to the eaves. (Mesh = 16 cm.)

Extractor fan, "Trojan", 25 cm. diameter, situated on the gable immediately under the ridge, extracting 31.14 cu.m. of air per minute.

The fan is brought into operation by a 'Sunvic' thermostat and relay when the shade temperature reaches 85°F (30°C), and will keep the inside temperature down to 95°F (35°C) under full

sunlight conditions. This control is irrespective of the outside temperature as the high humidity, which is maintained inside the greenhouse by soaking a 2 cm. thick layer of fine gravel down each side of the floor, effectively reduced temperature as the outside humidity drops with rise in temperature. It is thus possible for the outside temperature to be higher than 95°F and still maintain an inside temperature of 95°F.

The present extraction rate is not adequate and a fan double the capacity (giving one complete air change per minute) is indicated. The problem of an adequate air flow would be simplified by increasing the size of the gauze mesh but this would immediately facilitate the entry of insects.

3.2. The Soil Block Method of Seedling Cultivation.

The composition and use of soil blocks for plant raising have been given by Lawrence and Newall (1949). The method was first used in the study of Puccinia polysora by Storey in East Africa (1953) for cultivating single maize seedlings for inoculation with rust, but has not been tried for any purpose in West Africa, where it is necessary to use substitute materials and to modify the nutrient compositions of the blocks to suit the high temperatures encountered, and consequently the altered soil metabolism.

The problems involved in using the soil block method for

the cultivation of maize seedlings were:

a) to find a compost which might be standardised and was suitable for compressing into blocks, and

b) to provide adequate nutrients during the time the plants were to be retained in the blocks.

It was considered that three weeks was a suitable time from germination to discarding of the seedlings or their transfer to larger containers and the nutrients are calculated for this period. This is also suited to the practice of inoculating seedlings with rust ten days after germination and scoring 19 or 20 days after germination.

Compost materials.

a) Loam. In accordance with temperate zone practice, a search was made for a soil which corresponded to a medium clay loam. A local soil of the Egbeda series (Vine, 1954) was selected having the following percentage composition: 15.4% stones, 46.35% coarse sand, 28.1% fine sand and 10.15% silt and clay. The texture was medium, and the pH 5.1.

b) Humus. For making soil blocks, a humus fraction possessing organic material is necessary to prevent binding. Various humus materials of local origin were tried including rotted palm leaves (Elaeis guinensis Jacq.) manure, rotted sawdust and rotted vegetable refuse. For use with a sterilised soil a material is required which is itself sterile or can be sterilised without the release of undesirable toxic substances. For

this reason all the materials mentioned were discarded in favour of fresh mahogany sawdust. It is known that this material in the initial stages of decomposition is capable of immobilising nitrogen and the nitrogenous fraction of the fertilizer incorporated in the final compost was modified to overcome this difficulty.

c) It has been shown by Lawrence and Newall (Loc.cit.) that many of the sands used in compost making do not have angular grains and are too fine in texture for a good compost. This difficulty was not experienced and local washed river sand (silver sand) was used with success.

d) Nutrients. On the formulation of the basic composition of the block various levels of nutrients were incorporated, and these experiments are fully reported in the West African Maize Research Unit Memorandum No.3. (1954).

e) Lime. In field experiments on the Egbeda soil series (Vine, 1954) the only nutrients which gave responses were nitrogen and phosphate and little or no response to lime, although the soil is quite acid. A separate experiment was carried out on the addition of lime and this is also fully reported in Memorandum No.3 (1954).

f) Soil sterilization. The soil component alone of the compost was sterilized. The other ingredients were assumed not to possess a harmful microflora or fauna. The soil was sterilized in a G.E.C. soil sterilizer of 1 bushel capacity and 1 K.W. loading. Sterilization takes 60 minutes to bring the temper-

ature to 185°F and the current is then switched off and soil left inside for a further 10 minutes. The sterilized soil was spread out in a 3 inch layer to cool and was used immediately in mixing the compost which, after the addition of the fertilizers and lime, was watered to 75% of field capacity. The fresh weight of the blocks was 620 gms. when containing 17% moisture.

The Compost.

Soil blocks now in use have the following composition:-

7 parts sterilized black cocoa soil Egbeda series (Vine 1953)
3 parts mahogany sawdust
2 parts washed river sand
} by volume.

To each cubic metre of compost the following nutrients were added:-

Ammonium sulphate	1.36 kg.
Calcium superphosphate	1.36 kg.
Potassium sulphate	0.68 kg.
Ground Calcium carbonate	0.34 kg.

With this formulation seedlings of Zea mays can be grown satisfactorily under West African conditions for three weeks in the size of block used and experiments showed that these blocks can be readily prepared using local materials. Their use is now standardized in all rust inoculation studies in the greenhouse.

3.3. Inoculation Techniques.

Seedlings were grown singly in soil blocks. The soil

blocks were arranged in aluminium seed trays, 40 x 25 cm., each tray containing eight blocks. The trays were laid on 1 m. high metal trolleys to facilitate movement in the greenhouse. Several methods of inoculating seedlings with uredospores were tried and are briefly described below.

a) Dry dusting method.

Uredospores were removed from the infected maize leaves with a dry brush and dusted onto the upper half of the 1st, 2nd, and 3rd leaves ten days after germination of the seedling. Following dusting, the plants were lightly sprayed with water from an atomiser and covered with a cellophane hood for 36 hours. This method was modified after preliminary trials due to leaf blight infections appearing on the seedlings owing to the transfer of conidia of Helminthosporium turcicum Pass. and Cochliobolus heterostrophus (Dreschl.) Dreschl. from the mature maize leaves used to provide uredospores. Contamination was overcome by using stock cultures of rust grown on seedlings in the greenhouse.

b) Spore smearing method.

The upper half of the 1st, 2nd and 3rd leaves were lightly passed through the thumb and first finger to remove the bloom and then inoculated by spreading on a thin uniform layer of uredospores with the flat side of a scalpel. The seedlings were afterwards sprayed lightly with water from an atomiser and covered with a cellophane hood as before.

c) Spraying with a uredospore suspension.

A dense aqueous suspension of uredospores was obtained

by shaking pieces of heavily infected maize leaf in water. This suspension was then applied with a wide-nozzled sprayer, the quantity applied being just sufficient to cover the leaves of the seedlings with a uniform coating of droplets of spore suspension. The seedlings were then covered with cellophane as previously. Trouble was encountered with uredospores floating and clumping in the water and this was overcome by using 0.1% gelatine in distilled water as a suspending medium.

Results

The following points emerged from the various inoculation techniques employed.

a) The removal of cuticular wax by passing through moistened fingers prior to inoculation ensures a higher density of resultant uredosori.

b) Inoculation method (b) is not suitable owing to the mechanical damage of the leaf epidermis.

c) The first leaf of the seedling should not be used owing to great variability of reaction types between individuals of known genetic identity. The best infection court giving uniformity of reaction and maximum susceptibility is the upper third of the 2nd and 3rd leaves of the seedling.

d) Uredospore inoculum should not be taken from infected mature leaves collected in the field owing to the presence of other leaf pathogens. The standard practice now in use is to obtain spores from stock cultures of rust on seedlings; the original stock being obtained from single pustule isolations

and multiplied. This has eliminated contamination by leaf blights and the troublesome rust hyper-parasite, Darluca filum (Biv. - Bern. ex Fr.) Cast.

e) Subsequent to inoculation good infections require saturated humidity during the incubation phase. This is obtained by spraying the inside surface of the incubation hood with water from an atomiser prior to placing it over the seedling.

f) Polythene sleeving is used in preference to cellophane, the latter encouraging the growth of moulds (Stanton, 1951).

g) The maximum incubation time during which the seedlings may be kept covered is 48 hours under conditions prevailing at Ibadan. If this time is exceeded water-logging and die-back of the leaf tips occurs.

Standard inoculation method.

The following method is now in use for all greenhouse inoculations.

Seedlings are grown singly in soil blocks. The source of inoculum is obtained from rust cultures on the highly susceptible variety of maize, Lagos White, and this stock culture has been multiplied from an original single spore inoculation. The seedlings are inoculated on the 10th day after germination by firstly removing the bloom with moistened fingers and then dusting the upper third of the second and third leaves with uredospores. The seedlings are then lightly sprayed with distilled water from an atomiser and covered with a polythene hood for 48 hours.

This inoculation method is conveniently rapid for the testing of large host populations and gives uniformity of reaction of subsequent infections.

3.4. The Qualitative Assessment Method.

Seedlings are assessed on the 10th day after inoculation, by which time the pustules are mature. Storey and Ryland (1954) found that pustules took 14 days to mature in East Africa. The original qualitative assessment scale (Stanton and Cammack, 1953) was only provisional, based on the preliminary greenhouse observations during 1953, and has been modified after extensive use and now agrees closely with the scale employed by Storey and Ryland (1954a).

Class	+	
00	0	Host immune. No detectable effects on inoculated leaves.
0	0	Host showing high resistance. Hypersensitive flecking. No necrosis.
1	01	Host resistant. Uredosori minute, abortive and isolated, surrounded by sharply defined necrotic areas which are visible on the under-surface of the leaf.
2	1	Necrotic areas tending to merge into limited but distinct marginal chlorosis. Pustules small, rupturing the epidermis to show very few uredospores.
	X	
4	4	Host susceptible. Uredosori large, numerous in extensive clusters. Chlorosis indistinct or absent.

\+ Storey and Ryland Scale.

Storey and Ryland (loc.cit.) use Class X to define reactions lying between Class '1' and Class '4' of their scale. In the light of experience of greenhouse testing at Ibadan reactions in Class 'X' are unreliable in selection of breeding lines. Class 'X' reactions are only employed in critical studies of maize lines but for mass selection, and especially where doubt exists, they are classified as '4'.

Immunity, class '00', has not been found among varieties tested at Ibadan, and the true '0' reaction is confined to derivatives of Mexico 13 Acq. 95 (SLP 20 4A) received originally from the Rockefeller Research Centre.

3.5. The Relation between Juvenile and Adult Host reactions to Rust Infection.

The large number of acquisitions tested in the greenhouse, and the probability of their inclusion in the breeding programme made it necessary to test if juvenile qualitative reactions were also typical of the adult plant in the field.

The seedlings, grown in soil blocks were inoculated as previously described. After assessment the infected leaves were cut off and the plant transferred to the field. Adult assessment was done at the time of emergence of the tassel and again at the milk ripe stage. Typical juvenile and adult reactions of a selection of varieties are given in Table 16.

Table 16./

Table 16.

The relation between juvenile and adult host reactions to rust infection.

Progeny reference	Variety & pedigree	Seedling reaction	Adult Reaction	Increase in susceptibility.
2047	Mex. 1.	1	2	+1
1712	Mex. 2	4	4	
1065	Mex. June	4	4	
1738	Mex. 4 $S_1\#S_2$	2	4	+2
1658	Mex. 5	1	2	+1
1743	Mex. 7	2	4	+2
1568	Mex. 13	1	1	
810	Mex. 16 S_4	2	2	
1526	Mex. $17\#_2S_1$	2	2	
1704	Mex. 21 $S_1\#$	4	4	
1706	Mex. $22\#S_1$	2	4	+2
Acq.810	EAAFRO 53191	2	2	
" 811	EAAFRO 53193	1	2	+1
" 812	EAAFRO 53194	2	2	
" 813	EAAFRO 53197	1	2	+1
1844	Br.129 Haiti$\#S_1$	2	2	
1856	Br.132 Haiti$\#S_2$	1	2	+1
1849	Br.125 Haiti$\#S_1$	2	4	+2
1847	Br.130 Haiti$\#S_1$	2	2	
Acq.685	June-165	4	4	
" 684	June-141	4	4	
" 675	Tiquisate-10	2	2	
" 679	Yellow Tuxpan-12	4	4	

In some instances the juvenile reaction was found to be an indication of the behaviour of the adult in the field. The departures from identity are shown in the last column of Table 16. Without exception the fully susceptible reaction, class '4', in the seedling was also shown by the mature plant. Resistant reactions in the seedlings were not always an indication of resistance in mature plants and it has been observed that the level of resistance in the adult is often less than that displayed by the seedling and, in some cases, juvenile resistance gives a fully susceptible mature plant reaction.

SECTION 5.

Summary

1. Preliminary observations on the climate of West Africa showed that more rust was found in the humid coastal areas than in the drier northern regions.

2. A rust assay was designed to determine the extent and severity of the disease. Results showed maximum rust intensities along the West African coast and a gradual lessening of intensity towards the north. A relationship exists between the vegetation zones, taken as being an expression of all prevailing climatic factors, and the intensity of rust.

3. An automatic volumetric spore trap was used to determine the effect of the concentration of air-borne uredospores on the incidence of disease in the crop, both at different times of the year and over a period of years.

4. A study of the annual fluctuations in the uredospore content of the atmosphere suggests that a limiting concentration of viable uredospores, the 'primary epidemic threshold', is necessary to institute the epiphytotic.

5. The uredospore content of the atmosphere immediately above a maize plot has been studied and a correlation found between the concentration of uredospores and the incidence of disease in the crop.

6. The concentration of uredospores in the atmosphere at different/

different times of the year affects the pattern of natural infection in a maize population.

7. The factors contributing to the initial success of the epiphytotic in West Africa, and its subsequent decline are briefly described.

1. THE CLIMATE OF THE EPIPHYTOTIC AREA OF WEST AFRICA AND ITS RELATION TO THE INCIDENCE OF P. POLYSORA.

In order to determine the conditions favourable to the development and spread of the rust a survey was made of the climatic conditions prevailing in the epiphytotic area with the valuable assistance of the West Africa Meteorological Services.

Detailed accounts of the climate and vegetation of Nigeria are given by Brooks (1916 and 1920), Keay (1953) and Rosevear (1953), and of Ghana by Channey (1948) and Taylor (1952). The climate of West Africa in general is described by Kendrew (1941) and Harrison Church (1957) and these authorities have been drawn on in the following brief description of the climate of the epidemic area.

Winds in the epiphytotic area.

West African climates are controlled fundamentally by two dominant air masses, the dry continental air of the northerly Harmattan which extends from the Sahara to a maximum southward extent (in January) of 5°N, and the warm, humid mass of Tropical maritime air, the northeasterly Monsoon which reaches inland to 20°N. The two winds meet at the 'intertropical convergence line' (intertropical front, I.T.F.). In January the I.T.F. is farthest south at approximately 5°N, and the Guinea Coast is subjected to the influence of the dry Harmattan wind. As the sun returns the I.T.F. proceeds northwards and by March lies approximately along the 10°N latitude and the Harmattan is

replaced by the moist north-easterly monsoon, the latter being the prevailing wind for the greater part of the year. The Monsoon extends across the tropical belt of Africa, from coast to coast, during the months April to November.

Temperature and rainfall in the epiphytotic area.

The temperature and rainfall figures used in the text have been obtained from established meteorological stations along the coast of West Africa and from points in the interior along the known northern limit of the area affected by rust. The figures are monthly and annual mean averages taken over as many years as possible and in no case less than ten. Fig. VII shows temperatures prevailing in the affected areas during the year, and Fig. VIII the rainfall. Along the Guinea coast there is a marked gradation in temperature and humidity from the coast towards the interior. Temperatures are in the region of 80°F along the coast with small diurnal and annual ranges. Towards the interior mean temperatures are higher with greater diurnal and annual ranges. Fig. IX shows mean annual variations in temperature for three locations in Nigeria; Lagos on the coast, Zungeru in guinea savannah 400 miles north and Kano at the fringe of the sahel savannah 700 miles north of the coast. Along the coast temperatures rarely rise above 85°F, but may exceed 100°F in the northern interior. Rainfall, shown in Fig. VIII, is heaviest along the Guinea coast and decreases towards the interior. With the exception of a small area of coastal savannah, the Accra Plains in Ghana (Taylor 1952), the

annual rainfall on the coast exceeds 60" and is generally over 80".

Disease incidence and location.

In late 1952 and early 1953 the writer sent requests to all agricultural stations in West Africa for reports on the incidence of P. polysora on local maize. No quantitative method had yet been devised and reports were all based on casual visual observations. Table 17 lists the information obtained from stations on the coast and in the interior. Rainfall and temperature mean averages over a 10 year period are given for each station.

Table 17.

Preliminary reports of the severity of P. polysora in West Africa.

Station	Rainfall Annual Total	Temperature. Annual Mean	Annual Range.	Reported Rust Intensity
Dakar	22.7	78	9	Not known
Freetown	151.3	81	4	Moderate
Accra	27.4	80	6	Moderate
Lagos	71.4	81	5	Severe
Calabar	118.5	79	4	Severe
Duala	158.5	78	6	Moderate
Libreville	97.2	79	5	Severe
Bamako	44.0	83	13	Not known
Gao	9.3	86	22	No rust
Lokoja	48.4	81	7	Slight
Zungeru	45.4	81	8	Slight
Kano	35.1	80	18	No rust.
Zinder	21.6	83	21	No rust.

Conclusions were made with caution from these preliminary reports by reason of the casual method of observation and

individual interpretations of relative terms such as 'slight', 'moderate' and 'severe'. The following general observations were made.

I) The rust was more intense along the coast than in the interior.

II) An annual rainfall within the range 60 - 120" with an accompanying ambient temperature near 80°F favoured the rust. Where the rainfall was below, or in excess of, that range the rust incidence was less.

2. THE RUST ASSAY.

Casual reports of P. polysora listed in Table 17 gave a general indication of the extent of the disease. With the object of obtaining more precise information a series of assay plots was laid out in Nigeria and Ghana. With the co-operation of the Department of Agriculture, Dahomey, one plot was sited in that territory thus completing the link between Nigeria and Ghana.

Material and Methods.

Each assay plot consisted of 24 ridges each 25 m. long and 1 m. apart. Two maize grains were planted at ½ m. spacing along the ridge and thinned to 1 per stand after germination, thus giving 50 stands per ridge. Around the perimeter of the plot a 1 m. wide barrier was laid down consisting of benzene hexachlor-

mixed with sawdust and chopped grass as a carrier. This barrier was a precaution against pest infestation. In addition the inter-rows were also mulched and a liberal dusting of insecticide applied. A dressing of superphosphate fertilizer was made at planting time at the rate of 2 cwt. per acre and Ammonium sulphate applied at the rate of 1 cwt. per acre 30 days after germination by ringing the stands with circles of 6" radius.

From preliminary observations in the field, and using the quantitative assessment key described in Section 4.2.1., twelve varieties of maize covering the full range of susceptibility to P. polysora were chosen for incorporation in the assay plot. One variety was planted on each alternate ridge and the intervening ridges planted with the rust susceptible variety, Lagos White, to act as 'spreader lines'. The varieties incorporated in the assay are listed below.

Variety	Quantitative Rust Reaction
Mexico 1	1
Mexico 5	1
Mexico 13	1
Mexico 16	2
American Yellow Bounty	2
La Creole	2
Tsolo	3
Mexico 2	3
Mexico 17	3
Bulom Corn	4
Abakaliki Red Flour	4
White Tuxpan	4

All the assay plots planted in the first assay, 1953, are listed in column 1 of Table 18 and in addition two in Cameroons under British Trusteeship at heights of 4000 and 7000 feet

respectively. All plots were planted on the 10th April, or as soon after as local conditions permitted, in order to standardise planting time.

Each plant of each variety in all of the 27 plots was assessed for rust using the technique described in Section 4 on the 30th, 50th, 70th and 85th days after germination.

Table 18./

Table 18.

The relationship between vegetation zones and the incidence of maize rust, P. polysora, determined from 25 rust assay plots distributed throughout the territories of Nigeria and Ghana.

Territory and Vegetation Type	Rust Intensity. Plot mean	Zone mean	Time of planting in days after the 10th of April.
NIGERIA			
(a) Sudan Savannah			
(i) Maiduguri	0.0	0.5	81
(ii) Daura	1.0		58
(b) Guinea Savannah			
(i) Samaru	1.6	1.6	50
(c) Relic Rain Forest			
(i) Yandev	0.5		35
(ii) Abakaliki	2.7		20
(iii) Auchi	0.9	1.6	2
(iv) Nkwelle	2.4		25
(d) Rain Forest			
(i) Abak	2.9		8
(ii) Umudike	1.0		0
(iii) Ogba	1.6	2.8	10
(iv) Agege	4.0		16
(v) Ipokia	4.0		17
(vi) Ibadan	3.0		15
(vii) Efferun	2.7		
DAHOMEY			
(a) Rain Forest			
(i) Naiouli	3.5	3.5	?
GOLD COAST			
(a) Guinea Savannah			
(i) Wenchi	1.0	1.0	8
(b) Coastal Savannah			
(i) Pokoase	1.7		0
(ii) Mankessim	4.0	2.9	26
(c) Relic Rain Forest			
(i) Kpeve	1.6	1.6	4
(d) Rain Forest			
(i) Mampong	2.5		6
(ii) Sunyani	3.5		8
(iii) Aiyinasi	3.3		14
(iv) Bunsu	3.1	3.2	18
(v) Kumasi	3.5		18
(vi) Esiama	3.5		20

Results of the First Assay, 1953.

The varietal means from the 4 rust assessments were determined for each plot and from those the plot means were calculated. These are listed in Table 18. Inspection of the rust values confirmed the earlier casual observation that the rust was most intense in the coastal areas and decreased in intensity towards the north. The rust values of each plot were compared with local climatic factors and it was apparent that the amount of rust was related to temperature, rainfall and humidity. Climatic data was often scanty or totally lacking and it could not be determined which were the main controlling factors or whether in fact the incidence of rust was governed by the interaction of all factors.

It was decided to relate the rust values obtained in the first assay with vegetation zonations (Keay, 1953), the zones being an expression of all climatic and environmental factors in any one area, and the groupings obtained are given in Table 18. The zone means, Table 18, column 3, were obtained by taking the average of the plot means of all plots lying within each vegetation zone. In both Nigeria and Ghana the highest rust intensities were recorded in the coastal rain forest areas and in both territories the intensities decreased towards the north, in plots sited in transitional forest or grassland zones.

The first assay was subject to several errors, principally the differences in planting dates, which varied greatly owing to wide variations in the time of onset of the rains and other

problems of husbandry. Irrespective of their position, assay plots planted late showed considerably more rust than those planted earlier. An example of this is shown in the two assay plots sited in the coastal savannah zone of Ghana. Pokoase was planted on the 10th April, at the onset of rains and showed a mean rust value of 1.7. Mankessim, planted 26 days later, had a value of 4.0. The two plots were assessed by the same observer.

The assay was repeated in 1954, methods being identical to those used in 1953, and again obvious errors were introduced by the variation of planting time.

The Third Rust Assay, 1955.

From experience gained in the first two assays several modifications were made. The number of plots was reduced to 7, sited at the locations listed in Table 19. These sites were chosen principally because they were representative of all climatic types encountered in the maize growing areas of the two territories, and because adequate supervision could be given to the plots by trained observers. Spreader lines were omitted from the plots since they were considered unnecessary. Application of fertilizers and the use of insecticide were the same as in the assay.

In order to determine the effect of planting time on the incidence of rust three plots were planted at monthly intervals at each site. Since it did not prove possible to plant the plots of every series at isolation distances the three plots were planted adjacent to each other. The funds available did not

permit replications of the experimental design. The dates were chosen so that the second planting would coincide with the estimated onset of rains. The respective planting dates are given in Table 19.

Table 19.

The locations and times of planting of plots in the third rust assay, 1955.

Location of Plot.	Planting Dates 1st Planting	2nd Planting	3rd Planting
NIGERIA			
Samaru	9.5.55	6.6.55	4.7.55
Umuahia	15.3.55	12.4.55	9.5.55
Ibadan	15.2.55	15.3.55	12.4.55
Agege	15.2.55	15.3.55	12.4.55
GHANA			
Tamale	9.5.55	6.6.55	4.7.55
Kumasi	15.2.55	15.3.55	12.4.55
Asuansi	15.2.55	15.3.55	12.4.55

Results.

The rust intensities at each assessment date in each plot are given in Fig. X. The following results were obtained.

i) Time of onset of rust.

The staggered planting dates enabled the time of onset of rust to be determined at each location and in general it was

found that onset was much earlier in the south than in the northern areas. In the southern high rain forest areas rust first appeared as early as the 20th April on plots at Kumasi and Asuansi in Ghana and on the 29th April at Agege in Nigeria. In the northern guinea savannah areas the onset was late in both territories. At Tamale, in Ghana, rust first appeared on the third plot on the 12th August, the first two plots being free of rust. At Samaru in northern Nigeria the rust was first observed on the third plot on the 16th August. In the southern rain forest areas rust became established at the onset of the rains but in the northern savannah it first appeared two months after the start of the rains.

ii) Intensity of rust attack.

The amount of rust encountered in the different areas conformed with the infection pattern of the first two assays. In the plots sited in rain forest zones near the coast, where the ambient temperature is near 80°F with small diurnal and seasonal ranges and consistently high humidity, the rust was much more intense than in the northern savannah where ambient temperatures approach 90°F with large diurnal and seasonal ranges and low humidity. Rust values at maturity time, expressed as plot means, ranged from 3.6 - 3.9 in the south and from 0.9 - 1.9 in the north.

iii) Rate of increase of rust.

Irrespective of position the rate of increase of rust with time became more rapid as the season progressed, as shown by

the amount of rust observed at each successive planting date. In the plots in the southern rain forest the rate of increase of rust was greater than that in the northern savannah, especially after the 50th day when the plant enters into the reproductive phase.

iv) The annual spread of rust.

The pattern of spread was observed each year during the assay. In Nigeria first reports of the appearance of rust were always obtained from the south-west corner of the territory and from the region of the Niger delta. In Chana rust was first observed in the high rain forest area in the south-west. Towards the north of both territories reports of rust attack came progressively later with distance from the coast and the pattern was suggestive of inoculum from the initial source of infection being carried northwards by the S.W. monsoon and establishing infection on the later planted maize in the northern areas.

v) The distribution of rust intensity.

Information on the distribution of rust intensity was augmented by extensive observations throughout Nigeria and Ghana and the distribution of relative rust intensities is shown diagramatically in Text Fig. XI. It was not possible to obtain complete information from maize growing areas in the interior of Dahomey. The distribution of rust intensities was related to the different vegetation zones as had been suggested in the first assay and it was apparent that the incidence of

rust was controlled to an extent by the interaction of the climatic factors prevailing in the different areas as expressed by the vegetation type. The ranges of rust intensity encountered in the different zones are given below.

Rust Intensity.	Zone.
0 - 1.0	Sahel and Sudan savannah region. Mean annual temperature 82°F with an annual range of 20°F and large diurnal range. Low average R.H. Rainfall 25-35".
1.0 - 1.9	Guinea savannah region. Mean annual temperature 80°F with annual range of 10°F and moderate diurnal range. Low average R.H. Rainfall 40-45".
1.5- 3.5	Derived savannah region with relic rain forest. Mean annual temperature 80°F and annual range of 10°F. Small diurnal range. Humidity ranging from 70-95% R.H. Rainfall 45-50".
2.5 - 4.0	Lowland rain forest region. Mean annual temperature 80°F with small annual range of 7°F and small diurnal range. Constantly high humidity, 75-95% R.H. Rainfall 45-55".
3.5 - 4.0	Mangrove forest and coastal fresh water swamp region. Mean annual temperature of 80°F with very small annual range of 5°F and small diurnal range. Constantly high humidity 85-95% R.H. Rainfall 70-120".

The three rust assays described gave information on the extent of the disease and on the variations in intensity in the different climatic areas.

Having defined the extent of the disease attention was directed to the effect of air-borne inoculum, the viable uredospore, on the incidence of disease, and whether variations in the atmospheric concentration of inoculum affected the degree

of rust incidence in addition to the effects of variation in climate. The following experiments describe some aspects of the behaviour of the air-borne uredospore in relation to disease incidence.

3. THE ATMOSPHERIC UREDOSPORE CONTENT IN RELATION TO THE INCIDENCE OF RUST: STUDIES WITH AN AUTOMATIC VOLUMETRIC SPORE TRAP.

Knowledge of the atmospheric spore content is important in the study of the epidemiology of plant diseases. The atmospheric spore content has been estimated by many workers with different types of traps, principally exposed sticky surfaces in the form of planes or cylinders. Gregory (1950) has investigated the trapping qualities of exposed sticky surfaces, especially narrow vertical cylinders.

For a critical study of the behaviour of a plant pathogen, a trap having a high trapping efficiency and little variability is required. Johnson (1950) has shown the errors of exposing sticky surfaces and calculating the efficiency of the trapping surface based on a mean wind speed taken over the total period of exposure. Some workers (Gregory, 1951, 1952) and Gregory and Stedman (1953) have shown that most trapping methods do not give true values of the air spora and that few allow an accurate assessment of spore fluctuations with time. Several traps designed to increase the efficiency of impaction of spores on

the trapping surface have been described by Hawes, Small and Miller (1942) and by May (1945), the efficiency being increased by accelerating a narrow stream of air towards a sticky surface. May (loc.cit.) also stated the conditions necessary for a high collecting efficiency. The automatic volumetric spore trap designed by Hirst (1952) was constructed so that the spores passed through a narrow orifice directed into the wind and were impacted onto a sticky surface at a constant speed. The sticky surface moved past the orifice at a rate of 2 mm. per hour allowing estimates of the spore content at different times to be easily determined. Wind tunnel tests showed this trap to have a high trapping efficiency.

The following experiments were carried out at Ibadan during the years 1953-56 to determine the seasonal and diurnal behaviour of the epidemic and the relation of the atmospheric uredospore content to the incidence of disease in the crop.

Methods.

a) The operation of the trap. For purposes of determining diurnal and seasonal fluctuations in the atmospheric spore load the trap was sited beside a meteorological station in the middle of a 7-acre field of close mown grass (Cammack, 1955) and the orifice of the trap was positioned 2 m. above ground level. The field was situated in the middle of 850 acres of mixed annual and perennial crops including a large acreage of Zea mays, none of which was within 400 m. of the trap. For investigations on the relationship of the uredospore content of the atmosphere

to the incidence and trend of disease in the crop the trap was placed centrally within a 4.5 acre plot of maize with the orifice 3 m. above ground level.

Air was drawn through the orifice of the trap at the rate of 10 litres/ minute by a rotary vacuum pump which was housed in a modified Stevenson screen placed below the stand of the trap. The constant running of the motor in the high day temperatures caused slight overheating and excessive vaporization of oil in the pump which had to be frequently replenished. The air was passed through an asbestos-oil filter between the trap and the pump to remove dust and sand particles and prevent wearing of the vanes of the pump.

b) The recording of wind speed. Trapping efficiency varies with wind speed, owing to the suction rate through the orifice being constant and not altered with the wind speed to obtain isokinetic sampling. It was therefore necessary to obtain a constant record of fluctuations in wind speed. A sensitive, three-cup anemometer of the electrical contact type was mounted adjacent to the trap and at the level of the orifice and connected to an impulse recorder, each electrical contact activating a solenoid operating a pen which charted the wind speed. After each period of 3 minutes a second solenoid was activated which returned the pen to zero and moved the chart round to the next position. The pen then commenced to chart a vertical line parallel to the previous one, its total length representing the total wind speed during the 3 minute period. From the calibrated

chart the number of impulses could be read off and the wind speed obtained from a curve. Each spore count was corrected in accordance with the mean wind speed over a period of one hour before until one hour after the sampling time and corrections made based on calibration tests of trapping efficiency (Hirst, 1953) and the mean wind speed.

c) Preparation of adhesive slides. Spirit cleaned slides were first primed with a thin film of 'Solvar', a partially hydrolysed polyvinyl acetate, and allowed to dry. Several of the commercial 'Solvar' series were tried and the best found to be 'Solvar' 3515 which was least prone to wrinkling on drying out, a troublesome artefact in the spore trace. At the suggestion of J.M. Hirst (in correspondence) the use of a priming film was discontinued, and an adhesive alone proved satisfactory. Vaseline as an adhesive, which proved suitable in temperate climates (Hirst, 1953), could not be used at Ibadan due to the high day temperatures which caused distortion of the trace owing to the melting of the adhesive. A mixture of B.D.H. soft white paraffin with 1½% ceresin wax (congealing point 60°C) proved satisfactory and retained good optical properties. The slide was warmed and the molten wax dropped on until the surface was covered, the surplus then being drained off and the slide laid on a flat surface to set. Wax was then removed from strips 3 mm. wide down each long edge of the slide to facilitate insertion into the slide carrier of the trap.

d) Mounting of slides. Hirst,(1953) used a mountant consisting of 50 parts of 20% solution of 'Solvar', 25 parts lactic acid

and 25 parts of 6% phenol. As a probable result of the consistently high humidity at Ibadan this mountant would not set and remained fluid. The formula was modified to incorporate a higher percentage of 'Solvar' and a satisfactory mixture, which retained its clearing properties and set within a few days, consisted of 75 parts of 25% 'Solvar', 15 parts lactic acid and 10 parts 6% phenol. In cases where it was necessary to accelerate the setting time the slide was transferred to a desiccator one day after mounting.

e) Spore counts. Counting was done on a series of bench tally counters mounted on a base board and placed conveniently near the microscope. Six counters, mounted in two banks could easily be operated with one hand, leaving the other free for microscope adjustment. Where only one spore type was being counted a hand tally counter was found convenient. Daily mean concentrations can be quickly determined by counting 'long traverses', (Hirst, 1953), parallel to direction of slide movement, but corrections for the efficiency of the trap can then only be applied on the basis of the daily mean wind speed. For more accurate estimates at smaller time intervals, counts were made on 'short traverses', (Hirst, 1953), normal to the direction of slide movement at positions representing known time intervals and corrections applied for the efficiency of the trap using the mean wind speed from one hour before until one hour after the time represented by the trace. When using the short traverse method counts were made at 2-hourly intervals. A 100 μ wide trace was most

convenient for counting uredospores. All counts are expressed as spores/cu.m. of air.

3.1. The Diurnal Periodicity of Uredospores of Puccinia polysora in the Atmosphere.

In order to interpret the numerical results obtained with the spore trap it is necessary to know the pattern of diurnal fluctuation of the uredospore content of the atmosphere. It is also of value to know when peak concentrations occur during the day and samplings of short duration should be done during that period in order to minimise error.

The diurnal periodicity was obtained from slides exposed over the period April-June, 1954. Counts were made by the 'short traverse' method at 2-hourly intervals on a total of 53 slides exposed during that period. For purposes of comparing the pattern of the diurnal periodicity in Ibadan with that obtained with uredospores in a temperate climate the mathematical treatment is the same as that employed by Hirst (1953), periodicities being expressed as percentages of the peak geometric mean concentrations. Corrections for the trapping efficiency were applied to each count. The diurnal periodicity is shown in Fig. XII. The form of the curve is very similar to that for the uredospore group found by Hirst at Rothamsted, although the peak concentration obtained at Ibadan was not so distinct and were more protracted. Peak concentrations occurred between 12.00 and

14.00 hours and generally dropped sharply thereafter, though it was observed on some days where exceptionally dry conditions and strong winds prevailed during the afternoon, the value of the peak mean concentrations was often maintained until as late as 17.00 hours. Night values, with lower temperatures and long dew periods, were always low.

3.2. The Seasonal Fluctuation in the Atmospheric Uredospore Content: the Primary Epidemic Threshold.

The seasonal fluctuation was determined in order to follow the pattern of the air spora in relation to the seasonal incidence of rust on the crop. During the years 1954-56 a trap was run continuously in an exposed site at Ibadan at a distance of 700 m. from the nearest maize plot. Slides were counted each day by the 'long traverse' method, corrected for mean wind speed, and plotted in Fig. XIII. A direct relationship was observed between the air spore concentration with time and the availability of host plants. During the dry months December - March, spore values were as low as 0.2/cu.m. In early April, in accordance with local practice, the first season maize crop was planted and in the square mile around the trap a total of 200 acres was planted. Seven weeks later natural infection appeared on the maize and the air spora rose rapidly to a concentration of over 500/cu.m. in early June. This value was maintained until harvest in early July, thereafter dropping to less than 100/cu.m. by early September. The second crop is planted in

the first week of September and by 6 weeks later, in mid-October natural infection had appeared on the second crop and the recorded air spora had again risen to over 500/ cu.m. This value fell sharply immediately after harvest in mid-November and by December had fallen to less than 10/cu.m. This decline continued until January when the concentration was once more approximately 0.2/cu.m., this low value being maintained throughout the dry season and until early June when the following year's first crop was showing infection. The above figures refer to the year 1954. Observations during 1955-56 showed that whereas the mean values may vary considerably the form of the curve is the same each year and illustrates the large fluctuations in atmospheric spore content during the year.

The low concentrations during the dry months of January to March are interesting. Uredospores trapped during that period were at first thought to have come from maize trash from the previous season. The observation of rusted maize plots on river banks and compounds raised the possibility of a proportion of those spores being viable. This was checked by using 2% malt agar as an adhesive on the slides and during the dry season 1954-55 and again 1955-56 uredospores were trapped which commenced to germinate on the slide and which were positively identified as P. polysora. The concentration of such viable spores was very low, averaging 0.08/cu.m., but was significant in the respect that viable uredospores were present in the atmosphere throughout the year and substantiated the suggested

method of carry-over of the rust from crop to crop described in Section 3.

A second observation made on the results was that during the years 1954-56 natural infection was not observed on maize in the vicinity of the trap until the recorded spore concentration exceeded approximately 5/cu.m. To this arbitary value the term 'primary epidemic threshold' is given since it is suggested that this may be the minimum concentration of uredospores which, dispersed uniformly in the atmosphere, can successfully institute infection, and that values lower than that have an insignificant chance of alighting on a favourable infection court and instituting infection. This view was substantiated in the field by sequential plots of maize, planted at 10 day intervals throughout the year in isolation one from the other. Each year infection did not appear until early June when the spore concentration was within the range 5-10/cu.m. This 'primary epidemic threshold' is only applicable to the first crop each year since at the time of planting of the second crop the concentration is within the range 40 - 90/cu.m.

Continuous records of the uredospore content of the atmosphere have been used successfully each year in predicting the time of onset of the epiphytotic, and this onset has always coincided with calculated spore concentration of 5 or more uredospores/cu.m.

3.3. The Uredospore Content of the Atmosphere Above a Maize Plot from Planting until Maturity.

Preliminary to an experiment on the relationship of air spora immediately above the crop and the incidence of disease in the crop, the fluctuation in air spora at a height of 3 m. above ground level (i.e. immediately above the level of the crop) was determined.

A volumetric trap was put into operation on the 1st April, 1955, giving a continuous record of the air spora. On the 19th April 4.5 acres of maize were planted around the trap and from then until harvesting on the 12th July the numbers of uredospores of P. polysora in the atmosphere were determined at 5 day intervals. Ten, 100 μ wide traces were counted on the long axis of the slide and the daily mean concentration of uredospores/cu.m. determined. Counts were also made on the day prior to and the day following each date, and the mean averages plotted in Fig. XIV. On the 19th May, rust was first observed in the plot. Prior to this date the uredospore content was low, presumably coming from early planted and irrigated plots in the vicinity. The concentrations of uredospores began to rise from 5/cu.m. on the 19th May to 16/cu.m. by 50% tasseling stage of the maize population. Thereafter, spore numbers increased rapidly to a mean value of 194/cu.m. on 3rd July, the time of 50% death of the maize population. The rapid rise in spore numbers after tasseling time corresponds with the observed increase in the rate of spread of rust on the upper part of the

maize plant after it has entered into the reproductive phase. After the 3rd of July spore numbers dropped slightly but maintained a high level until the end of the experiment 98 days after planting. The peak of the curve corresponds to the time at which the maize population is becoming senescent and it would be expected that spore production would be greatly reduced and finally cease at that time. Spore concentration did, however, remain high and it is suggested that the concentrations represented by the dotted part of the curve are largely those released from debris.

3.4. The Atmospheric Uredospore Content in Relation to the Incidence of Rust in a Maize Plot.

The same site was used in this experiment as in the previous one. On the 19th April, 1955, 4.5 acres of maize were planted at 0.5 m. spacing between plants on the ridge and 1 m. between ridges. The volumetric trap was installed in the centre of the plot, with its orifice 3 m. above ground level.

Around the trap 100 plants were marked at 2 m. intervals in the form of a grid and each leaf on every plant labelled and numbered on emergence. On the 19th of May the first rust appeared on the plot, one plant showing pustules. From then until the termination of the experiment a continuous record of the air spora was obtained. The nearest other maize to the experiment was 700 m. south-east and it was assumed that the density of inoculum from this source reaching the trap would be

insignificant in relation to the density of uredospores released from the maize surrounding the trap. At five day intervals, subsequent to the appearance of rust on the crop, the numbers of uredospores trapped were counted. Counts were made in 10, 100/u traces along the long axes of the slides and calculated as the number of spores per cubic metre; this number being the average over a 24 hour period. Counts were also made on the day prior to and the day following the assessment date and the mean average determined.

The incidence of rust on the marked maize stands was also measured at 5 day intervals. The amount of rust was expressed as the density of uredosori per unit leaf area. Pustule counts were made with a transparent grid constructed from a 3" square of perspex divided by scored cross lines into 0.5" squares. The graticule was placed over each leaf of the plant in turn and a total pustule count made. Results were expressed as the mean average pustule density per sq. inch of total leaf surface. The respective values for pustule density and spore load are given in Table 20.

Table 20/

Table 20.

Values of the mean atmospheric spore load and mean pustule density at 5 day intervals. Values below the double line are those recorded after 50% death of the maize population.

Date	Spore load S/metre^3 (values corrected for efficiency of trap)	Pustule Density P/sq.in.
3.6.55	10.1	1.5
8.6.55	14.2	5.0
13.6.55	24.2	29.1
18.6.55	36.4	54.7
23.6.55	125.4	82.1
28.6.55	194.0	99.8
3.7.55	194.0	121.5
8.7.55	146.3	126.5
13.7.55	100.1	136.5
18.7.55	98.0	84.0
23.7.55	98.0	50.0

Values of the mean spore load were plotted against mean pustule density for each assessment date as shown in Fig. XV. The correlation coefficient between mean pustule density and spore load is 0.92 and the formula is

$$y = 0.15 (\pm 0.07)x + 12.4 (\pm 15.42)$$

This regression is significant at the 0.1% level. The errors of slope and constant are given for $t(5)$ at the 95% probability level.

The values after 50% death have been inserted in the graph but fall outside the limits of the regression line. This may be

explained by the high level of uredospore discharge from dead maize observed in experiment 3.3.

The correlation only holds within a certain period beyond which it is subject to error. At the time of onset of rust on the maize surrounding the trap the number of uredospores discharged is not significantly greater than the number of spores trapped which have come from other sources. Anomalous results are also obtained due to a progressive decline in pustule formation at senescence of the host and the abnormal discharge of spores from dead tissue. The relationship has been found to hold only until a time corresponding to 50% death of the host population.

Work on the relationship is continuing in an attempt to devise an assessment method whereby the degree of rust incidence in a large acreage of maize may be determined by sampling the air spora for short periods within the crop.

3.5. The Atmospheric Spore Load in Relation to the Pattern of Natural Infection.

In order to obtain data on the effect of various atmosphere spore loads on the pattern of natural infection of a maize population two plots of maize were planted at two times of the year during 1956.

The site chosen was in isolation from other maize, the nearest plot being 250 m. down wind. The plots consisted of 250 stands in a block of 15 x 15, planted at 0.5 m. spacing on

the ridges with 1 m. between the ridges. The first plot was planted on 7th April at which time the atmospheric uredospore content was 1.3/cu.m., and the second on the 3rd September, when the atmospheric spore content was 97.1/cu.m. Each plot was assessed daily from time of germination for the appearance of rust, and thereafter at 10 day intervals. At each observation date the infected plants were marked on a plot diagram. In the first planting two plants showed infection 49 days after germination. The number of plants showing infection at 10 day intervals after first appearance of rust are given in Table 21. The numbers of infection doublets (Van der Plank, 1946) were also counted. Thirty days after the first infection all the plants were rusted. In the second planting one plant showed infection after 41 days. Twenty days later all plants were infected.

On doublet analysis of the pattern of infection in the first planting there was strong evidence of secondary, or 'neighbour' spread at the 20th day assessment after first appearance of rust. This was confined to areas around the initial infections and was most likely due to infections arising from the first generation of spores liberated from these plants. The spread was most noticeable down the direction of the prevailing wind.

Table 21./

Table 21.

An analysis of the pattern of infection in two plots of maize planted on 7.4.56 and 3.9.56 respectively.

Onset of Rust (days after germination)	Observation Dates (days after onset of rust)	Number of plants showing infection	Doublets expected d_{ex}	Doublets observed d_{ob}	S.E.	Spore load S/cu.m.
		First Planting				
49	10	16	1.1	1.5	1.21	16.3
	20	67	19.6	31	4.47	20.8
	30	All plants infected.				47.0
		Second Planting				
41	10	49	10.5	7.5	3.24	209.0
	20	All plants				221.0
	30	infected.				270.0

Evidence of neighbour spread was not observed in the second planting. On the 10th day after first appearance of rust 49 plants showed rust and these were distributed at random throughout the plot. On the 20th day all plants were infected.

Inspection of the uredospore content of the atmosphere in Table 21 shows that at the time of first appearance of rust in the first planting the concentration of air-brone potential inoculum was low, 16.3/cu.m., and throughout the period of the experiment did not rise above 47/cu.m. In the second planting the spore concentration was very high at the time of first appearance of rust and remained in excess of 200/cu.m. throughout the experiment. These levels of inoculum determined the

pattern of infection in each plot. In the first planting the low level of air spora initiated very few primary infections, distributed at random. Ten days leter, on analysis of doublets, evidence was obtained of down-wind neighbour spread from these initial infections which, in the 10 day interval, would have been capable of initiating infection by secondary spread. In the second planting the higher concentration of air spora had initiated 49 infections distributed at random order throughout the plot by the 10th day after the first infection had been observed on one plant. The distribution of these infections disproved the possibility that they were secondary infections from the first generation of spores discharged from the initial source and that they were, in fact, mostly primary infections from air-borne spora. Ten days later, by which time secondary spread would have been apparent, all plants showed infection.

This experiment illustrates the extreme caution which must be observed in investigating the pattern of spread of rust in a plot and in order to interpret results a knowledge of the uredospore content is required. The effect of the variation of the concentration of air-borne inoculum with time has been discussed with reference to the form of disease gradients from a point source (Cammack,1957).

4. THE EPIPHYTOTIC IN WEST AFRICA.

4.1. The Annual and Secular Fluctuations of the Epiphytotic.

In temperate climates most diseases have a mono-modal peak in their annual periodicity, characterised by an initial phase with a low level of infection, thereafter rising rapidly to a peak followed by a sudden decline. Most curves of annual fluctuations of plant diseases have been constructed from assessments of the incidence of disease on the host and this method was used in preliminary observations on P. polysora, but later substantiated by observations on the fluctuations in the atmospheric uredospore content. The relationship between the incidence of rust on the host and the uredospore content of the air immediately above the crop is described in experiment 3.4 of Section 5, and this correlation is sufficiently good to justify using the air spora as an expression of the incidence of rust.

The annual fluctuation in the uredospore content of the atmosphere is shown in Text-Fig. XIII and shows the bi-modal form of the curve. This is fully explained in experiment 3.2 of Section 5. The bi-modal curve, as opposed to the normal mono-modal form is readily explained by the availability of hosts during the year. During the months January-April the spore content is very low, coming mainly from debris and to a lesser extent from infected plots of irrigated maize. The steep rise of the curve corresponds to the onset of infection of the first annual maize crop and the curve falls after its harvest. The second peak again coincides with onset of infection on the

second annual crop.

As far as is known there is no secondary host in the life cycle of P.polysora nor is it known to possess a perennating mycelium. The simple relationship between the incidence of the uredospore and the availability of hosts in the annual periodicity tends to support the theory that the rust perennates in the uredospore stage.

The secular periodicity, during the period 1949-56 has been determined as far as possible from figures of estimated losses in yield in Nigeria and Ghana. A localised study has also been made at Ibadan, Nigeria, on continuous quantitative records of the uredospore content of the atmosphere.

Estimated losses in yield listed in Table 3 are subject to large error but suggest that the disease rose rapidly to a peak of destructiveness after its introduction into West Africa, thereafter declining and reaching a constant value. On examination of peak mean concentrations of uredospores in the atmosphere at Ibadan the figures obtained were indecisive and fluctuated greatly from year to year within the period of sampling.

Table 22.

Peak mean concentrations of uredospores in the atmosphere two metres above ground level during the years 1954-57.

Year	Peak Mean Concentration	
	June	November
1954	97.1	109.0
1955	187.3	212.4
1956	41.0	187.4
1957	37.1	?

These variations can be attributed mainly to differences in the averages of maize plant in the vicinity of the volumetric trap each year and also to changes in prevailing climatic factors and these variations with time invalidated the use of the uredospore content of the atmosphere as a measure of the incidence of disease over a period of years. Quantitative assessment of standard assay plots (Section 5. 2), planted each year during the period 1953-56, showed a consistently high level of infection, class 4.0 of the quantitative scale, during 1953-54. The following two years showed a decline to 3.1 and 3.3 respectively. No quantitative records are available prior to 1953. Whereas the host plants used had genetic identity each year many ~~other~~ environmental factors were variable and the results could not be termed significant.

It is difficult to obtain quantitative statistical data on the virulence of rust over a period of years, but observations on the pattern of spread of the disease in Africa combined with reports of economic losses suggest that the disease followed the classic pattern of a progressive epidemic, explosive in the initial years, quickly reaching a peak of virulence and thereafter declining.

4.2. Conditions Favourable to the Establishment of the Epiphytotic.

a) The availability of the host. When a pathogen enters a new area the chances of it causing an epiphytotic are greatly

increased when the host is prevalent and extensively cultivated. The ease with which a cereal rust may spread in a large area of host is adequately illustrated in the annual spread of wheat rust from Mexico to Canada each year. In West Africa Zea mays is grown extensively in all areas along the Guinea Coast (Stanton, 1957) and on the introduction of P. polysora in 1949 the resultant spread was rapid, presumably by chain infections instituted by uredospore inoculum carried along the coast by the prevailing wind during the maize growing season, the North-easterly monsoon. Evidence from reports of first appearance of P. polysora in the various areas of Africa (Table 4) suggests that the eastward spread resulted from air-borne inoculum instituting a chain of infection across the maize-growing belt from West Africa through the Belgian Congo to East and South-East Africa and islands in the Indian Ocean.

b) The susceptibility of the host. Prior to the appearance of P. polysora in West Africa it is known that the rust was confined to the Americas. The host and potential pathogen, in respect to West Africa, were, therefore, in isolation and as a result the host possessed no inbred resistance or tolerance. The rust on arrival encountered a completely new 'Land race' of the host.

c) The aggressiveness of the pathogen. The virulence of the pathogen in its initial stages was also conditioned by the isolation of the rust and its host as also was the high level of susceptibility of the West African maize population. Each was confined to its own floristic region and in the Americas the

rust, in the course of time had attained disease saturation of the host with resultant equilibrium. All available evidence suggests that P. polysora was not present in Africa prior to 1949 which would adequately explain its aggressiveness in the years immediately following its introduction.

d) The reproductive capacity of the pathogen. If any pathogen is to be successful in establishing an epiphytotic within the host population it must possess high reproductive capacity and infection potential. An attempt was made to estimate the uredospore discharge of P. polysora.

Individual pustules were selected on potted maize plants grown on the greenhouse and protected from draughts. From the emergence of the pustule until death of the host leaf, uredospores were removed from the pustule with a cyclone spore trap at two times of the day, 08.00 hours and 18.00 hours. The spores were inpacted onto the surface of 1.0% gelatine solution in the collecting chamber of the trap. After thorough mixing the concentration of uredospores was determined with a 'Thoma' blood cell counting slide and the total number determined. With this method it was estimated that one uredosorus, growing on the rust susceptible variety Lagos White, liberates 1,500 - 2,000 spores each day for a period of 18-20 days from emergence. The resistant variety Mexico 5 was found to liberate 600 - 1,150 uredospores each day for the same period. Each uredosorus on a susceptible host is therefore capable of producing $2.7 - 4.0 \times 10^4$ spores during the life span. On this basis a plant of the variety

Lagos White infected at the 7th week of growth could discharge 14×10^9 uredospores prior to its death. A large population similarly infected would discharge sufficient spores to saturate the air with inoculum and infect every plant of neighbouring populations.

In addition to large numbers of inoculum a low numerical threshold of infection is necessary in establishing a disease in epiphytotic proportions. In Section 3. 5 it is found that a significant number of infections can be obtained by single spore inoculations under known optimum conditions. Each uredospore alighting on a suitable infection court and subjected to the correct environmental conditions is a potential source of infection.

The rhythm of successive generations of uredosori, nine days in P. polysora, combined with the speed of production of spores and rapid germination within 4 - 12 hours all contribute to the exceptionally high reproductive capacity of the rust.

e) The dissemination of spores. In progressive epiphytotics it is the maximal range of germinable spores which is important. In experiments on the dispersal of inoculum from a point source (Cammack 1957) the uredospores of P. polysora behave typically under conditions of normal turbulence (Gregory, 1945). There is, therefore, no theoretical limit to the dispersal distance of uredospores of P. polysora. It is evident on studying the spread of the rust in Africa that chain infections occurred across the African Continent. Since a continuous maize growing area extends

across the Continent such a chain of infection in short steps would be possible and the ease of air-borne dispersal would not be offset by the short viability of the uredospore and its intolerance of low temperatures (Section 3) which would be encountered in the upper atmosphere during long range dispersal.

f) The environment. Maximum chance of infection subsequent to a spore alighting on an infection court is dependent on the prevailing environmental conditions being suitable. Optimum or near-optimum conditions for natural infection exist along the West African Coast (Section 5. 1) and the temperatures and humidity prevailing in that area are near the optima for uredospore germination found in the laboratory (Section 3. 4). Where less favourable climatic conditions prevail the intensity of rust is not so great. The relation of climatic factors to disease incidence is fully described in Section 5. 2.

The factors briefly discussed above influence the severity of the epiphytotic, both individually and collectively. P. polysora was introduced into a new land race of Zea mays in a favourable environment. The large areas of susceptible host, the virulence and high reproductive capacity of the pathogen and its ease of dissemination, and the near-optimum conditions for spore germination and infection resulted in the typical progressive epiphytotic which reached its climax in 1953, four years after its introduction, and thereafter began to decline.

4.3. Conditions Responsible for the Decline of the Epiphytotic.

During the years 1954-56 the disease has declined in severity and this is again typical of the form of a progressive epiphytotic.

a) Disease saturation. The first contributing factor to the decline in severity is the saturation of the host population with disease. In the early years of the epiphytotic a vigorous system of natural selection of the host was in operation. The most susceptible local varieties in West Africa were so severely attacked that in many instances grain yields were nil. Those varieties which showed a measure of resistance were adopted by the farmer and extensively planted. Without human intervention a limiting level of disease saturation would have been reached in time as has already taken place at the place of origin of the rust.

b) Human selection of the host. The result of natural selection in the first instance followed by human intervention is a reduction in the disease proneness of the host. It was assumed that resistance to the disease would be found at the centre of origin of the rust where it had presumably been present for a long period of time and host-parasite equilibrium had been established. This proved correct and many varieties of Central and South American origin were imported into West Africa and some of those showed a high level of resistance to the local form of P. polysora. After field selection these are now being

extensively distributed.

c) Decrease in virulence of the pathogen. The process of elimination of susceptible individuals by natural selection has been greatly accelerated by breeding techniques and the introduction of rust resistant varieties. The pathogen is consequently confronted with a resistant host population. A consequence of this is a decrease in the numbers of uredospores produced (Section 5. 4.2 (d)) with the result that the concentration of potential inoculum in the atmosphere is decreasing and the chances of successful infections is being progressively reduced.

In addition to the purely quantitative consideration described above evidence is available that the pathogen is now undergoing a reduction in qualitative virulence. Two varieties, originally acquired in 1952, and whose genetic constitution has been retained by repeated selfing, were again tested qualitatively for rust susceptibility in February 1957. The following reactions were obtained.

	Reaction Type.	
	June 1953	February 1957.
10 White Tuxpan Pro. No. 257	4	2
15 Tsolo Pro. No. 327	4	2 +

Since the seedlings used in both tests had known genetic identity it may be concluded that the reduction in susceptibility is due to a reduction in the virulence of the pathogen. This process will continue until a lower limiting value is reached between the inbred resistance of the host and the virulence of the pathogen.

GENERAL DISCUSSION.

The work described in the previous sections is a general consideration of Puccinia polysora in West Africa with special reference to Nigeria. The various aspects of the work undertaken were dictated by the terms of reference of the West African Maize Research Unit laid down at the inception of the Scheme in 1952. A new disease appeared in a new environment and the preliminary approach, the observational phase, consisted of a study of the extent and severity of the disease in West Africa and the methods employed were such as to obtain a rapid assessment of the problem. On the completion of the primary observation phase, attention was directed to a more critical study of the disease in relation to environment, and this second phase consisted of the rust assay and uredospore germination studies.

A general idea of the pathogen having been gained, the work was then directed towards control measures. Chemical control is not mentioned in the previous sections since, either the several sulphur, copper or mineral compounds applied gave no control, or, if control was obtained, the concentrations and rates of applications necessary proved phytotoxic and uneconomical. It became apparent at a very early stage that control of the American corn rust could only be obtained by breeding resistance. This resulted in the greenhouse studies described in Section 4, which have special reference to the construction of a qualitative assessment key and techniques of seedling inoculation.

Finally, a knowledge of the quantitative behaviour of the rust in the field was essential. Quantitative evaluation of the rust was approached from the aspect of the atmospheric uredospore content and for this purpose an automatic volumetric spore-trap was installed. This trap was the first ever to be used in a tropical environment and it was found that nearly all temperate climate techniques had to be modified as a consequence of the high ambient temperatures prevailing at Ibadan. Some of these modifications are briefly described in Section 5 and have since been adopted by subsequent workers using similar traps in other parts of the tropics.

It must be stressed once more that the approach to this new problem until the present time has been purely fundamental and that it is only now, after a general understanding of the behaviour of the disease in its environment has been obtained, that more specific research projects can be planned. Several points of interest which have emerged from this study of Puccinia polysora are briefly discussed below.

1. The World Distribution of possible forms of P. polysora.

Until 1941 Puccinia polysora had not been recorded on Zea mays. Between the years 1897, when Underwood first described the rust as a new species, and 1941, P. polysora had been identified on a total of 37 specimens from Central and South America, but all were species of Tripsacum. On review of the lists of cereal pathogens compiled during the period in the Americas no mention of any unidentified Puccinia species can

be found. It is evident that a considerable amount of work on maize pathology was carried out and it is most unlikely that any specimen of Puccinia polysora on Zea mays would have been overlooked. As far as possible this has been checked by the writer on Zea mays specimens supplied by the Arthur Herbarium and the Commonwealth Mycological Institute and P. polysora was not found on specimens of Zea mays collected prior to 1941. This raises the question whether or not the form of P. polysora observed on Zea mays at that date arose as a mutant of the Tripsacum form, suddenly appearing on the closely related genus Zea. In the West Indies P. polysora was observed on maize in 1947 for the first time, whereas infections on Tripsacum spp. had been reported casually some years previously. At the present time both genera are affected by rust but unfortunately no cross-inoculation studies have yet been done and it is not known if the two rusts are identical. In 1945 living material of Tripsacum laxum was introduced into Nigeria from Trinidad as a potential cattle fodder. This material was reported on the quarantine certificate as being heavily infected with Puccinia polysora. Clonal material of this original introduction was obtained by the author and repeated attempts to infect it by inoculation with uredospores of P. polysora from Zea mays grown at Ibadan failed. Until cross inoculations have been carried out in the West Indies and other parts of the world affected by the rust no final conclusions can be made but, on existing evidence, it appears that the rust on Zea mays is not

identical with that on Tripsacum spp.

The analysis of the size frequencies of samples of uredospores from different areas of the world has shown two main size groups and it is interesting that these two groups correspond to the two-directional spread of the rust from its area of origin. Work at present in progress on the effect of environment on uredospore size shows that light intensity, temperature, host nutrition and the degree of resistance of the host, all affect uredospore size under controlled conditions at Ibadan. Environment alone, however, cannot account for the highly significant difference obtained between uredospores from South East Asian material and those from the African Continent. The mean size of the several samples taken from each of these areas was remarkably uniform, as illustrated in the text, and yet the various samples were subjected to much greater variation in environment throughout both areas than was induced experimentally at Ibadan. The question of the possible existence of geographically isolated races is still unanswered, but the writer has recently gained the assistance of several workers in different areas of the world and six typing lines of maize have been sent to twenty-seven locations in S.E. Asia, West Indies, Central America and Africa. Qualitative reactions of the host both in the greenhouse and the field will indicate the existence or otherwise of races.

2. The life cycle of P. polysora in West Africa.

Extensive searches, both in the Caribbean area and in West Africa have failed to find aecidial and pycnidial stages of the rust and, on the basis of available evidence, P. polysora may be termed an autoecious Hemi-form. Both uredo- and teleuto-stages are present on Zea mays throughout the areas of the world affected by the rust, and more especially in the tropical montane areas. However, the teleuto-stage is very rare. Repeated attempts, both by Dr. H.H. Storey in Kenya and by the writer in Nigeria have failed to induce germination of teleuto-spores. Work recently begun at Ibadan on the structure of the teleutospore nucleus shows that it is apparently dissociated and dispersed in fresh material. During germinations studies no visible changes took place in the nucleus when subjected to the several physical and chemical stimuli and, on the basis of observations by other workers, it would be expected that the nucleus would contract prior to active division. It is the writer's opinion that the teleuto-stage is vestigial in the life cycle of P. polysora and that it has been suppressed owing to the success of the uredo-stage in the tropical environment.

3. The rust and the West African climate.

The rust assay conducted in Nigeria and Ghana showed that the severity of rust attack is principally governed by two main factors - temperature and humidity. Vegetation zones were at first adopted as a provisional grouping in relation to rust

incidence. Keay's classification for Nigeria is now generally accepted and the vegetation zones are a true expression of the interaction and summation effect of all prevailing climatic factors. This system has been retained and extended to other parts of West Africa affected by rust and the relationship holds true. It can also be generally applied to all other areas of the world in which P. polysora occurs. A more accurate evaluation of the effects of temperature and humidity on rust severity was obtained from the laboratory experiments on uredospore germination described in Section 3. These experiments, when related to the results of the assay, have shown that near optimum conditions exist for uredospore germination along the West African Coast. This, in conjunction with the results of studies on the density of uredospore production, the low infection threshold and the highly susceptible host population, has provided an explanation of the success of the rust in the West African environment.

The short viability of the uredospore, as determined in experiments at Ibadan, is noteworthy in the respect that, in conjunction with the absence of a secondary host, it suggests a possible means of control by instituting a 'closed season' each year during which time no maize would be grown. The four months long dry season each year would be sufficient but, as explained in the text, a great deal of 'out of season' maize is grown in irrigated sites during that period and these plants act as 'volunteer' hosts. The extent and nature of the

territories involved does not make this method of control possible.

4. Disease assessment.

It became apparent at an early stage that mechanical and chemical methods of control would prove ineffective and attention was then directed to the construction of a qualitative assessment key to assist the Plant Breeding section in establishing genetic resistance to the rust. A key to the qualitative assessment of rust on seedling maize was first devised in 1953 and later revised in 1955 when more experience had been gained. The second key, described in Section 4, is very similar to that in use by the East African Agriculture and Forestry Research Orgainisation in Kenya. It is essential that the two scales should be standardised since considerable interchange of genetic stocks takes place; but the two scales are not identical principally due to the different climates prevailing in the two areas and their effect on seedling reaction. It has been found, however, that no discrepancies have arisen in the assessment of seedlings in Kenya and Nigeria using the respective keys.

The quantitative assessment key described in Section 4 proved successful and has now been adopted for use by the Departments of Agriculture in West Africa which are co-operating with the Maize Research Unit. It has the advantage of being easy to use and providing a direct visual comparison which is most important when used by unskilled observers.

5. The atmospheric uredospore content.

The automatic volumetric spore trap has provided valuable information on the diurnal and annual fluctuations in air-borne inoculum, foremost among the observations being the determination of a 'primary infection threshold'. Preliminary work on the prediction of the time of outbreak of rust by means of the air spore load has been encouraging and it is hoped to extend this method to forecast outbreaks of rust and the expected severity of attack.

Preliminary experiments have shown that within certain limits a relationship exists between the incidence of rust in a crop and the concentration of air-borne uredospores immediately above the crop. This study is at present being developed and it seems likely that a method will be devised by which, under known climatic conditions, an evaluation of rust incidence in large acreages of maize can be made with a portable model of the volumetric trap, using short sampling times. On a long term basis the constant use of the trap has given information on the form of the epiphytotic over a period of years.

6. Future Policy.

The solution to the problem of control of P. polysora in West Africa, and all other affected areas, lies in the establishment of a resistant population of maize. A source of resistance has been found in maize varieties from Central and South America and in addition many Caribbean varieties can be classified as high yielding tolerants. Fortunately, many of those resistant

varieties possess good combining ability and the grain characters of the local, rust-susceptible maize varieties will be bred into the resistant lines to accommodate local preferences in grain types. Several synthetic varieties are now in production which give double the local yield, and which are environmentally suitable for use in West Africa. If the present strain of P. polysora in West Africa remains stable, and mutation does not occur, the maize growing areas should be saturated with a resistant maize population within the next three years. The chance of the appearance of new strains is lessened, though by no means eliminated, if a secondary host of P. polysora is absent, as appears to be the case.

Appendix 1.

A PRELIMINARY LIST OF MAIZE FUNGI AND DISEASES IN NIGERIA

Chytridiaceae
Physoderma maydis Miyabe, on culms, outer sheaths and leaves.

Mucoraceae
Rhizopus spp., on germinating maize grains.

Sphaeriaceae
Cochliobolus heterostrophus (Dreschl.) Dreschl., on leaves.

Hypocreaceae
Gibberella zeae (Schw.) Petch., on husks and male inflorescences.

Pucciniaceae
Puccinia polysora Underw., on leaves and stalks.
Puccinia sorghi Schw., on leaves.

Ustilaginaceae
Ustilago zeae (Beckm.) Ung., on all aerial parts.

Thelephoraceae
Corticium solani (Prill. and Delacr.) Bourd. and Galz., on cobs.

Agaricaceae
Marasmius sp., on leaves.

Fungi Imperfecti.

Phomaceae
Botryodiplodia theobromae Pat., on ears.
Darluca filum (Biv. - Bern. ex Fr.) Cast., (hyperparasitic on rusts).
Diplodia macrospora Earle, on ears and leaves.
Phoma insidiosa Tassi, on husks.

Moniliaceae
Aspergillus spp., on germinating maize grains.
Penicillium spp., on germinating maize grains.
Cephalosporium acremonium Corda, on germinating maize grains.

Dematiaceae
Curvularia lunata (Wakker) Boed., on leaves.
Helminthosporium turcicum Pass., on leaves.
Nigrospora oryzae (Berk. and Br.) Petch, on leaves and ears.
Nigrospora sphaerica (Sacc.) Mason, on leaves and ears.

Tuberculariaceae
Fusarium moniliforme Sheld. (Macro and micro-conidial forms of *Gibberella fujikuroi* (Sawada) Wollenw., on old husks and male inflorescences.

Sterile Mycelia
Sclerotium rolfsii Sacc.

Other Diseases

Leaf stripe: Chlorophyll deficiency.

Leaf spotting: Chlorophyll deficiency.

Pellucid ring spot: Cause unknown.

Leaf streak: Virus.

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KEY TO LOCATION OF ASSAY PLOTS.

1. Maiduguri
2. Daura
3. Samaru
4. Yandev
5. Bambui I - 4,000 ft.
6. Bambui II- 7,000 ft.
7. Abakaliki
8. Umudike
9. Abak
10. Nkwelle
11. Auchi
12. Ogba
13. Efferun
14. Ibadan
15. Ipokia
16. Agege
17. Naiouli
18. Kpeve
19. Mankessim
20. Pokoase
21. Wenchi
22. Mampong
23. Sunyani
24. Kumasi
25. Esiama
26. Bunsu
27. Afyinasi

PLATE I.

Puccinia polysora Underwood. Uredosori on *Zea mays* L.

x $\frac{2}{3}$.

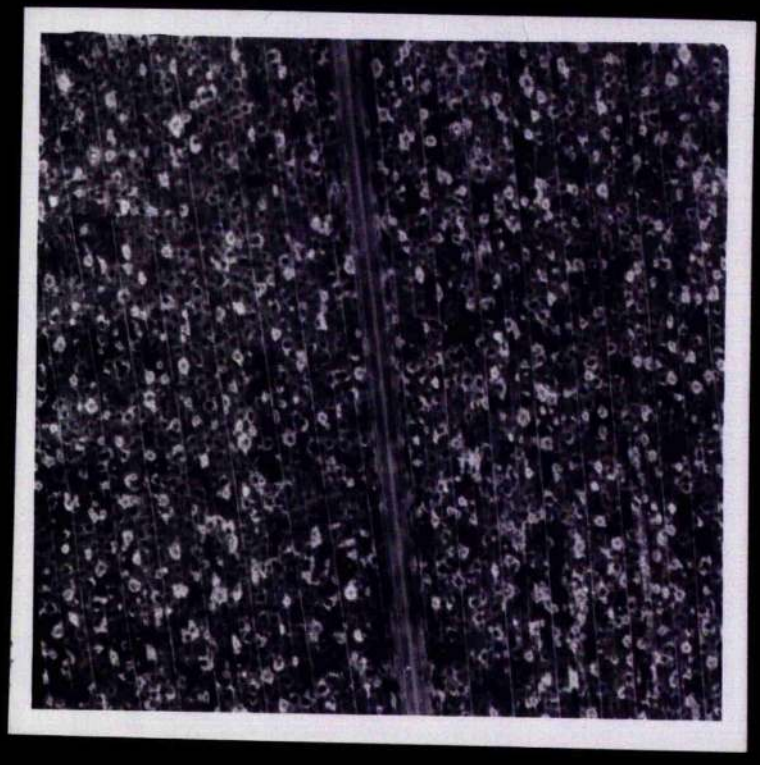


PLATE II. Figs. I and II.

Fig. I. The mean size distribution of samples of uredospores of *P. polysora* from different areas of the world. A = Philippines and Christmas Island; B = U.S.A.; C = Africa and West Indies; D = Borneo.

Host Key: ■ = *Euchlaena mexicana*;
▲ = *Tripsacum laxum*;
● = *Zea mays* .

Fig. II. Class frequency histograms of the dimensions of five samples of uredospores of *P. polysora* from different areas of the world.

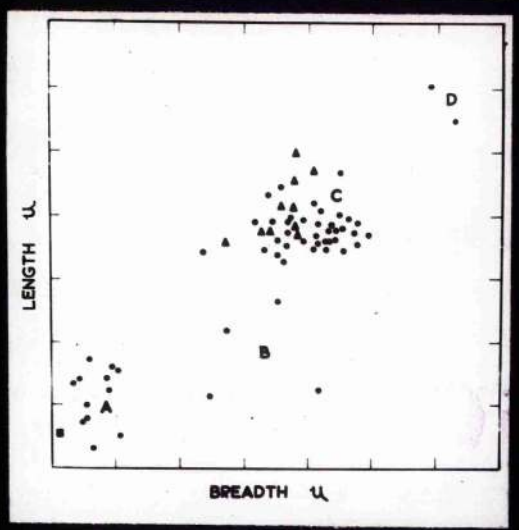
A = Philippines

B = Florida, U.S.A.

C = Trinidad

D = Nigeria

E = Borneo


LENGTH μ
BREADTH μ
A
B
C
D

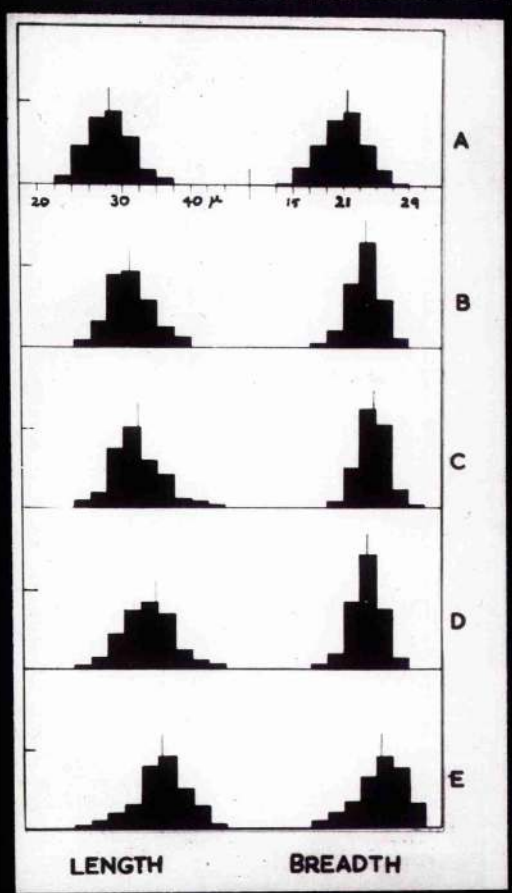

A
B
C
D
E
20
30
40 μ
15
21
29
LENGTH
BREADTH

PLATE III. Fig. III.

Fig. III. The percentage germination of uredospores of P. polysora at various constant temperatures and saturated relative humidity.

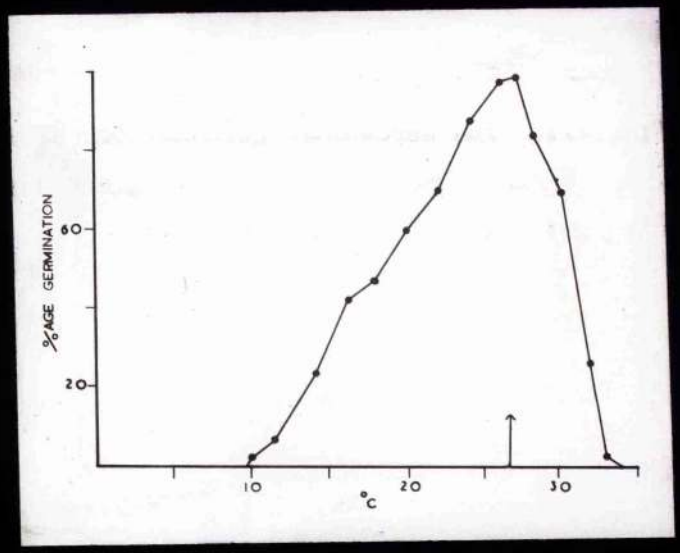

% AGE GERMINATION
80
20
10
20
30
°C

PLATE IV. Figs. IV and V.

Fig. IV. The quantitative rust assessment key showing the five degrees of rust intensity, 0 - 5.

Fig. V. The quantitative rust assessment key in use showing the method of direct comparison of rust intensities.

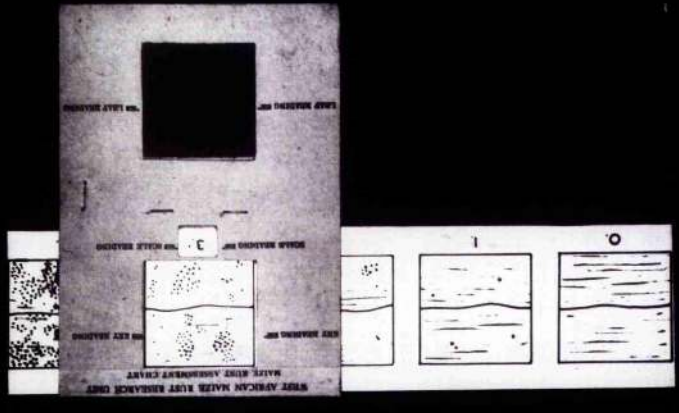

0
1
3

PLATE V. Fig. VI.

Fig. VI. A classification of maize varieties according to the class frequency of degrees of resistance to Puccinia polysora.

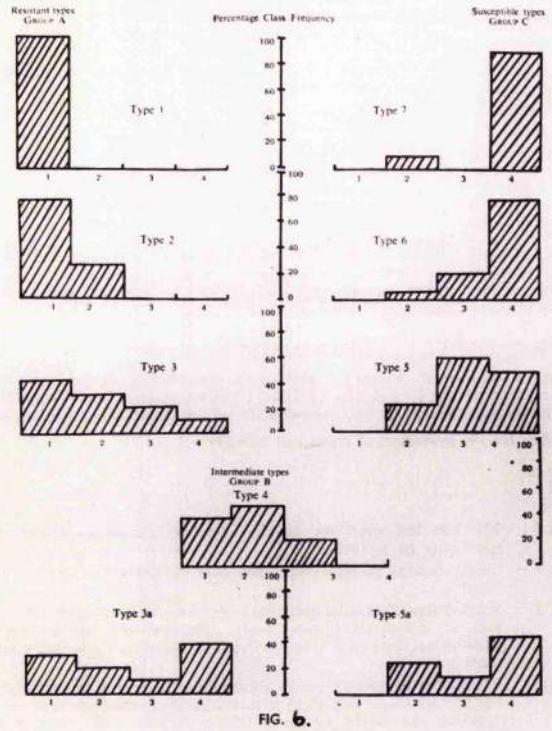


FIG. 6.

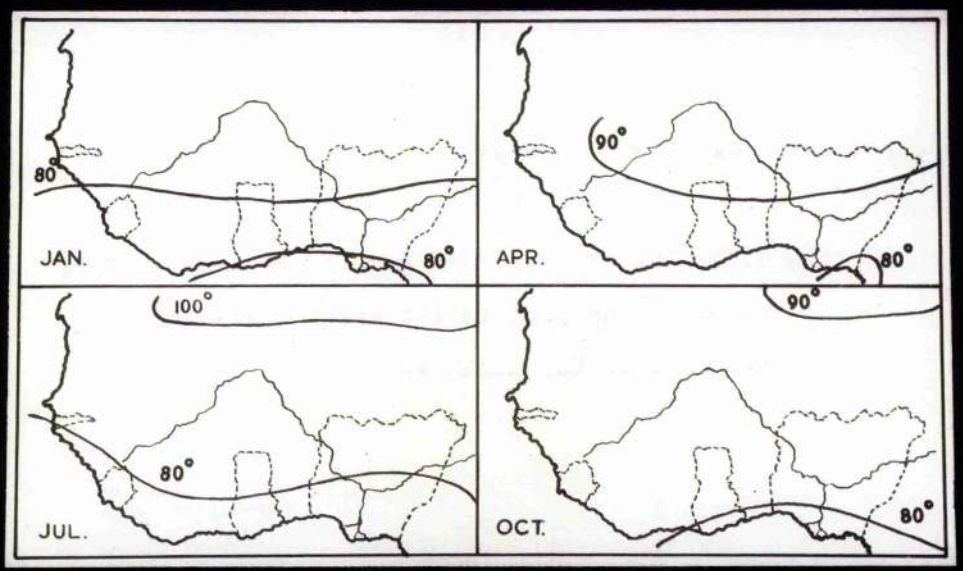
A classification of maize varieties according to the class frequency of degrees of resistance* to *Puccinia polysora*.

* As measured by frequency of pustule formation.

PLATE VI. Figs. VII and VIII.

Fig. VII. The mean temperatures prevailing at four times of the year in the area of West Africa affected by P. polysora.

Fig. VIII. The annual precipitation in West Africa. The isohyets shown are constructed in each case from the means of records over a period of at least ten years.


80°
JAN.
80°
90°
APR.
80°
100°
80°
JUL.
90°
OCT.
80°

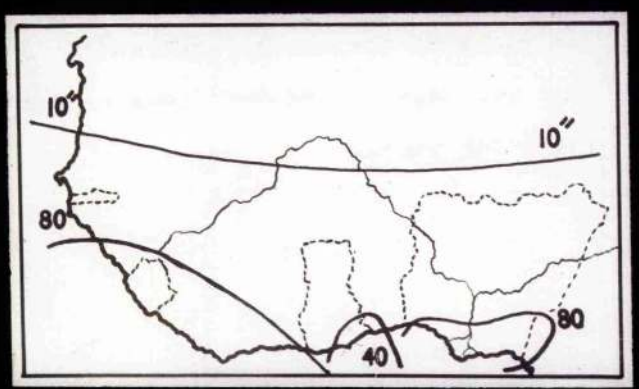

10"
10"
80
80
40

PLATE VII. Figs. IX and X.

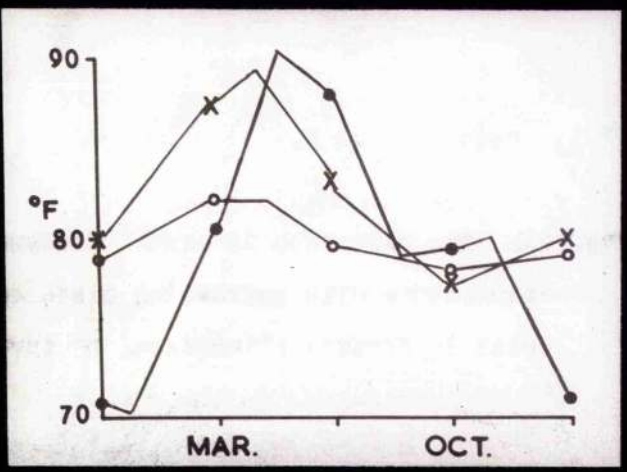
Fig. IX. The variation in annual fluctuations in temperature with increasing distance from the coast in Nigeria illustrated by three locations:

—O— = Lagos, on the coast;

—×— = Zungeru, 400 miles north;

—●— = Kano, 700 miles north.

Fig. X. Rust intensities in relation to vegetation zones in Nigeria and the Gold Coast during 1955. Assessments of incidence of rust made at four growth stages on each of three planting dates.



	VEGETATION ZONE	LOCATION	FIRST PLANTING	SECOND PLANTING	THIRD PLANTING
GOLD COAST	GUINEA SAVANNAH	TAMALE			
GOLD COAST	RAIN FOREST	KUMASI			
GOLD COAST	RAIN FOREST	ASUANSI			
NIGERIA	GUINEA SAVANNAH	SAMARU			
NIGERIA	RELIC RAIN FOREST	UMUAHIA			
NIGERIA	RAIN FOREST	IBADAN			
NIGERIA	RAIN FOREST	AGEGE			

PLATE VIII. Fig. XI.

Fig. XI. The distribution of relative rust intensities in Nigeria and the Gold Coast.

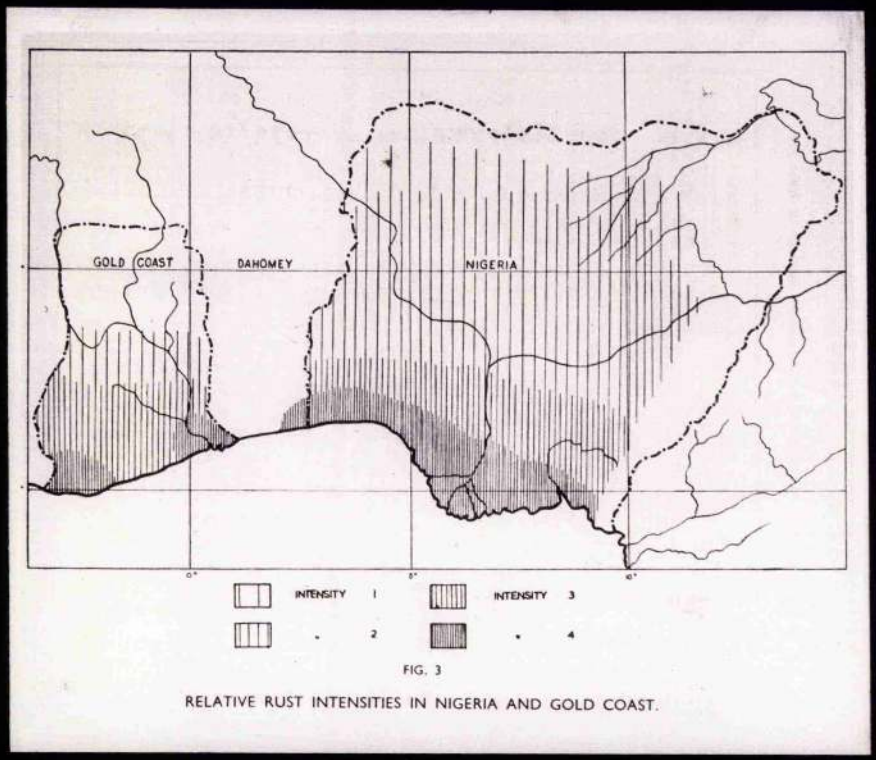


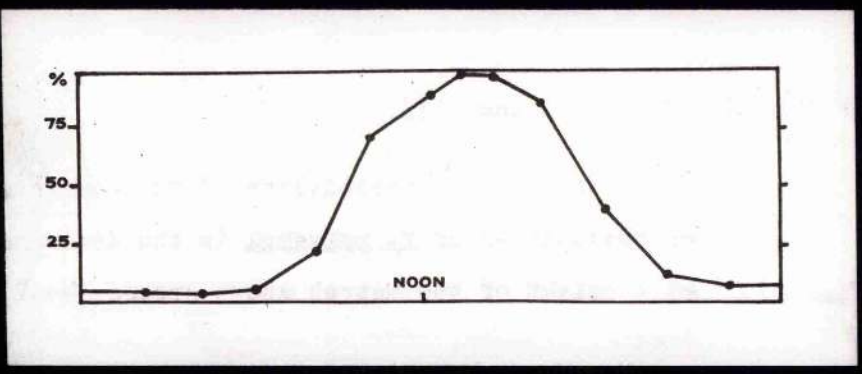
FIG. 3

RELATIVE RUST INTENSITIES IN NIGERIA AND GOLD COAST.

PLATE IX. Figs. XII and XIII.

Fig. XII. The diurnal periodicity of the concentration of uredospores of P. polysora in the atmosphere at a height of two metres above ground level at Ibadan, Nigeria.

Fig. XIII. The seasonal fluctuation in the concentration of uredospores of P. polysora in the atmosphere at a height of two metres above ground level at Ibadan, Nigeria. Arrows denote the normal times of planting of the two crops of Zea mays each year.


%
75
50
25
NOON

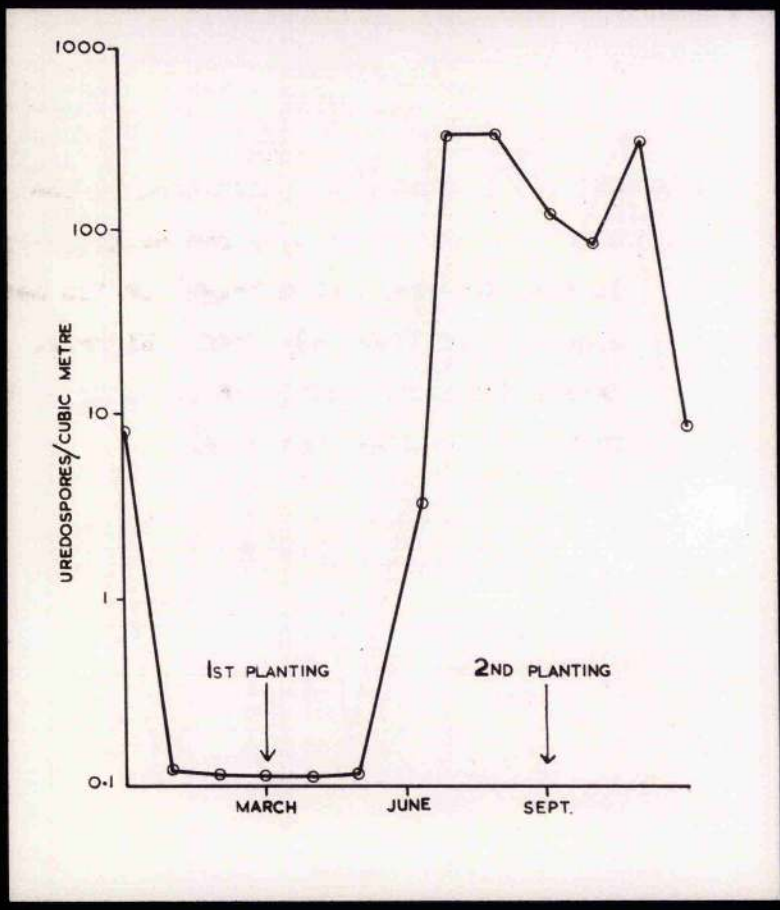
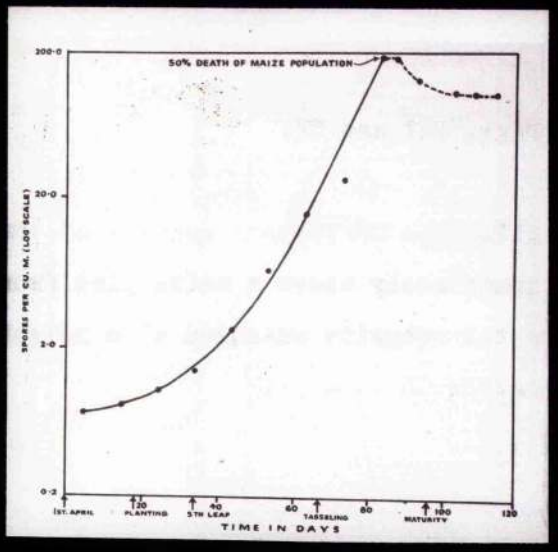
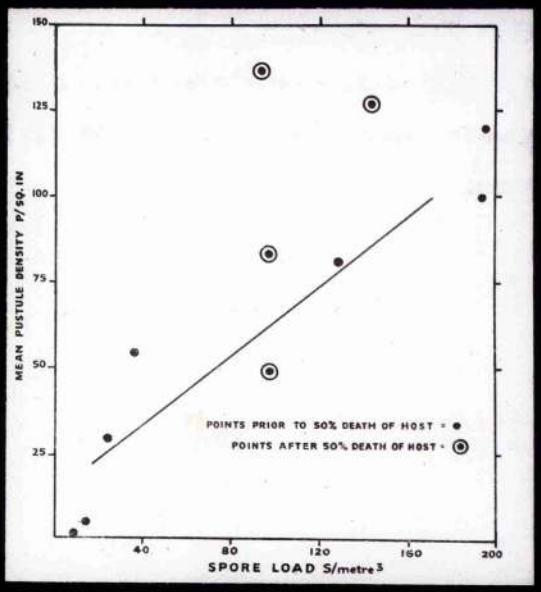

1000
100
10
1
0·1
UREDOSPORES/CUBIC METRE
1ST PLANTING
2ND PLANTING
MARCH
JUNE
SEPT.

PLATE X. Figs. XIV and XV.

Fig. XIV. The uredospore content of the atmosphere immediately above a maize plot from planting until maturity measured at a height of three metres above ground level.

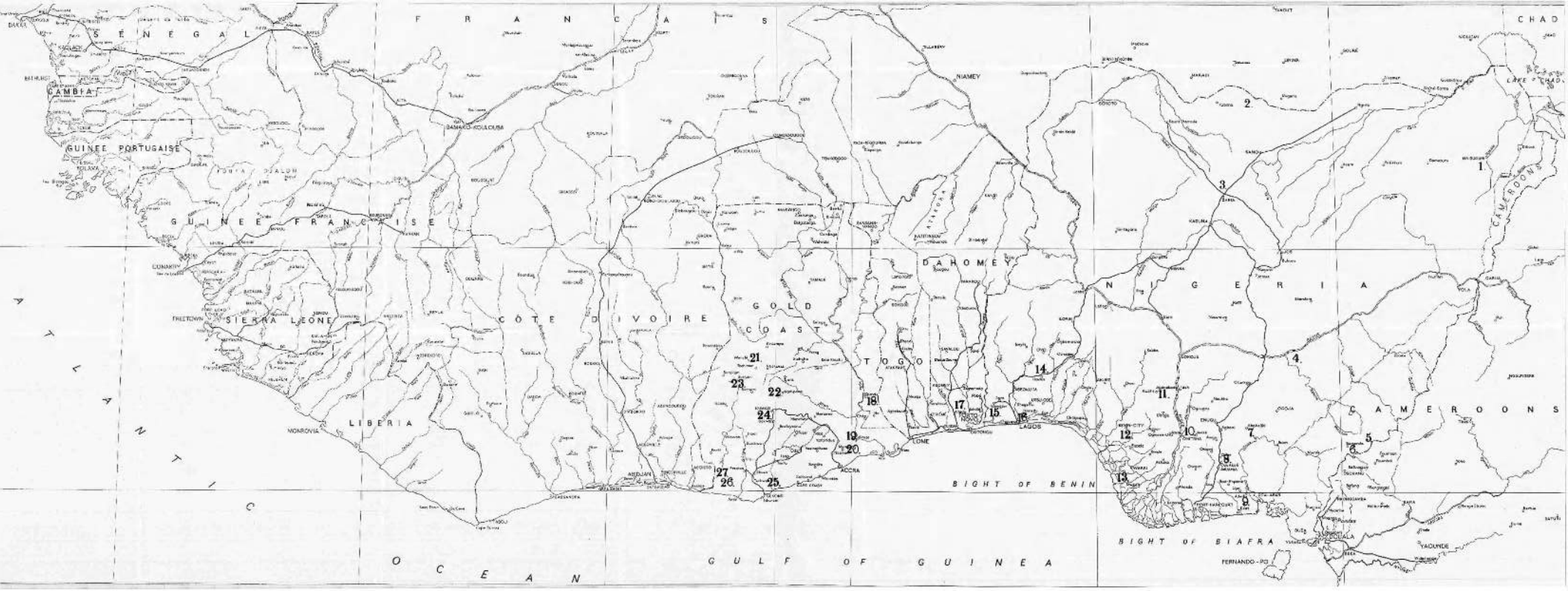
Fig. XV. The relationship between the uredospore content of the atmosphere three metres above a maize plot and the incidence of rust in the crop.


50% DEATH OF MAIZE POPULATION
SPORES PER CU. M. (LOG SCALE)
200·0
20·0
2·0
0·2
1ST. APRIL
120
PLANTING
40
5TH LEAF
60
TASSELING
80
100
MATURITY
120
TIME IN DAYS


MEAN PUSTULE DENSITY P/SQ. IN
150
125
100
75
50
25
POINTS PRIOR TO 50% DEATH OF HOST = ●
POINTS AFTER 50% DEATH OF HOST = ⊙
40
80
120
160
200
SPORE LOAD S/metre³

KEY TO LOCATION OF ASSAY PLOTS.

1. Maiduguri
2. Daura
3. Samaru
4. Yandev
5. Bambui I - 4,000 ft.
6. Bambui II - 7,000 ft.
7. Abakaliki
8. Umudike
9. Abak
10. Nkwelle
11. Auchi
12. Ogba
13. Efferun
14. Ibadan
15. Ipokia
16. Agege
17. Naiouli
18. Kpeve
19. Mankessim
20. Pokoase
21. Wenchi
22. Mampong
23. Sunyani
24. Kumasi
25. Esiama
26. Bunsu
27. Aiyinasi


SENEGAL
FRANCAIS
CHAD
GAMBIA
NIAMEY
BAMAKO-KOULOUBA
GUINEE PORTUGAISE
FOUTA DJALON
GUINEE FRANCAISE
DAHOMEY
NIGERIA
CAMEROON
SIERRA LEONE
FREETOWN
CÔTE D'IVOIRE
GOLD COAST
TOGO
LIBERIA
MONROVIA
ABIDJAN
LOME
ACCRA
LAGOS
BIGHT OF BENIN
BIGHT OF BIAFRA
GULF OF GUINEA
ATLANTIC OCEAN
FERNANDO-PO
YAOUNDE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
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